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Original Article

An experience with 124 cases of fanconi anemia: clinical spectrum, hematological parameters and chromosomal breakage analysis

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Abstract: Background: Fanconi anemia is an inherited bone marrow failure syndrome characterized by somatic abnormalities and an increased predisposition to malignancies. Objective: To determine the clinical spectrum and evaluate the hematological parameters as well as highlight diagnosis by chromosomal breakage analysis of Fanconi anemia patients. Material and Methods: A total of 124 patients were diagnosed as having Fanconi anemia from August 2014 to May 2020 at Armed Forces Institute of Pathology, Rawalpindi, Pakistan. Clinical details, somatic abnormalities, radiological findings, lab parameters and result of chromosomal breakage analysis were noted and analyzed. Results: One hundred and twenty four (14.29%) were diagnosed as having Fanconi anemia (FA) on chromosomal breakage test. Median age was 09 years 06 months. Male to female ratio was 1.9:1. Six of these patients exhibited mosaicism and were classified as FA mosaic. Somatic abnormalities were detected in 74 (59.7%) patients; the most common being skeletal abnormalities and short stature. Conclusion: Chromosomal breakage analysis is a cost-effective method for diagnosis of Fanconi anemia. Early diagnosis is pertinent for proper treatment and long term prognosis.

Keywords: Fanconi anemia, chromosomal breakage test, DNA repair

Introduction

Aplastic anemia is a disorder characterized by bone marrow failure manifested as pancytopenia and a hypo cellular bone marrow [1]. Inherited bone marrow failure syndromes constitute a significant number of patients presenting with aplastic anemia [2]. These disorders account for significant clinical burden and are associated with considerable morbidity and mortality [3]. The most common of the inherited causes of aplastic anemia is Fanconi anemia [4].

Fanconi anemia, first described in 1927, was named after the Swiss pediatrician Guido Fanconi, who described a familial form of aplastic anemia in three brothers with somatic abnormalities [5]. Fanconi anemia is an inherited autosomal recessive disorder characterized by a defect in DNA repair resulting in genomic instability and manifested as bone marrow failure, predisposition to malignancies and an increased sensitivity to certain cytotoxic therapeutic agents [6, 7]. Fanconi anemia is caused by mutations in a number of genes involved in the Fanconi Anemia (FA) pathway that is responsible for DNA repair during DNA replication [8]. The most commonly seen mutations are in FANCA, FANCC and FANCG genes [9].

This disorder is characterized by somatic abnormalities, hematological derangements and predisposition to malignancies [10]. The disease is genetically heterogeneous and clinically variable [11]. Pattern of somatic abnormalities and disease course are different in different patients, thus warranting chromosome breakage analysis to confirm clinical suspicion [12]. The underlying abnormality is a defect in DNA repair that renders extreme sensitivity to DNA cross-linking agents [13]. This is the basis of the laboratory screening test which assesses the chromosomal breakage after exposure to DNA crosslinking agents like diepoxybutane

(DEB) and mitomycin C (MMC) [14]. Definitive diagnosis is by molecular methods employing Fanconi anemia gene sequencing to identify the genetic defect definitively as well as to screen family members to identify potential HSCT donors [15, 16]. However, facility for molecular testing is limited and it is an expensive procedure, thus, chromosomal breakage analysis being readily available and cost-effective remains the test of choice for diagnosis especially in resource constraint countries.

This disorder is unique from aplastic anemia and early diagnosis is pertinent to ensure proper treatment [17]. Management is threefold; hematopoietic stem cell transplant for bone marrow failure, management of complications of any of the somatic abnormalities and long term follow up to monitor for any malignancies [18]. Consequently, clinical suspicion and prompt diagnosis is of crucial importance, not only for treatment but also for long term management of these patients, as these patients require regular monitoring for hematological and non-hematological malignancies [19]. Another important aspect is that Fanconi anemia is unique from other causes of aplastic anemia as Fanconi anemia patients require significantly reduced doses of chemotherapy during conditioning for hematopoietic stem cell transplantation [20].

Pakistan is a region where consanguineous marriages are the norm; therefore, autosomal recessive disorders are more frequent. However, no data is available from our population. Hence, we have conducted this research with the rationale to study Fanconi anemia patients, evaluate the spectrum of clinical findings, assess hematological parameters and analyze results of chromosome breakage analysis. Armed Forces Institute of Pathology is a tertiary care referral center with a fully developed Cytogenetic Department. This is the largest study from our region highlighting the frequency of this disorder as well as different clinical features that will help clinicians to diagnose these patients timely to ensure proper management and better outcome.

Material and methods

Study design

This cross-sectional study was conducted in the Department of Haematology and Cytogenetic, Armed Forces Institute of Pathology, Rawalpindi, Pakistan from August 2014 to May 2020. Armed Forces Institute of Pathology, Rawalpindi, Pakistan is a tertiary care referral facility catering to patients referred from different parts of the country. Patients from allied hospitals as well as distant urban and rural regions are referred here as it is the only cytogenetic facility in the northern part of the country.

Ethical approval

The study has been conducted in accordance with the principles of Declaration of Helsinki, after approval from Institutional Ethical Review Board AFIP Rwp (Ref No. Cons-05/READ-IRB/14/27). All patients and parents/guardians were elaborately appraised about the study and written informed consent was taken. Confidentiality and anonymity was maintained.

Patient selection

Suspected patients of Fanconi anemia with cytopenia, suggestive clinical history and presence of somatic abnormalities and/or positive family history referred to Armed Forces Institute of Pathology, Rawalpindi, Pakistan were recruited. All patients were Pakistanis, of Asian origin, belonging to all ethnic groups including Punjabi, Balochi, Sindhi, Pastoon, Kashmiri and patients from Gilgit-Baltistan. Control samples were taken from phenotypically normal age and gender matched healthy individuals. Siblings and first degree relatives of patients were not taken as controls. Patients having any chronic disorder or on any medications were not taken as controls.

Inclusion and exclusion criteria

All patients having a clinical suspicion of Fanconi anemia, based on cytopenias, somatic abnormalities and/or a positive family history; of all ages and both gender, were included in the study. Patients who had failed culture on cytogenetic analysis were excluded from the study. Siblings and first degree relatives of patients were not taken as controls. Patients having any chronic disorder or on any medications were not taken as controls.

Consent

All patients were elaborately appraised about the study and written informed consent was taken.

Clinical and hematologic parameters

Detailed history, inclusive of clinical symptoms, any etiological factor, family history and history of consanguinity was noted. Treatment and transfusion history was documented. Physical examination was performed. Clinical signs as well as any somatic abnormalities were noted. Radiological investigations, including X-rays for skeletal abnormalities and abdomen-pelvic ultrasonography for any urogenital abnormalities were carried out.

2.5 ml of peripheral venous blood was collected in EDTA. Complete blood counts were performed on automated hematology analyzer Sysmex XE-5000 and degree of cytopenias was assessed. Peripheral smears were prepared, stained with Leishmann and examined under Olympus light microscope. Anemia was defined as hemoglobin <10 g/dl, thrombocytopenia as platelet count $<100\times10^9$/l and neutropenia as ANC $<1.5\times10^9$/l.

EDTA samples were incubated at 37°C with equal amount of New Methylene blue and smears for reticulocyte count were prepared. Reticulocytopenia was defined as <0.5%. Bone marrow examination was performed. Bone marrow aspirate slides and trephine biopsies were examined to assess bone marrow cellularity. Bone marrow cellularity <25% was considered hypo cellular, 25-40% as moderately cellular and >40% as normal cellular.

Chromosomal breakage analysis

3 ml peripheral blood samples were collected in sodium heparin for chromosome breakage analysis. Mitomycin C (MMC) stock solution was prepared by adding 4 ml sterile water to 2 mg vial of MMC (Sigma-Aldrich). Two cultures were initiated for each patient - (A) 72 hour PHA stimulated culture in RPMI 1640 medium (Caisson Lab), (B) 72 hour PHA stimulated culture in RPMI 1640 medium (Caisson) with 200 ul of Mitomycin C (MMC) stock solution. Similarly, two cultures were set up for the control samples. Cells were harvested after 72 hours after colcemid (BDH Chemicals) treatment during the last 45 min. Four microscope slides were prepared for each culture and were stained with Giemsa. The slides were coded to reduce the element of bias. A total of 50 metaphase cells per subject were analyzed and scored for chromosome number and for the number and type of chromosome and chromatid aberrations, according to the International System for Human Cytogenetic Nomenclature (ISCN). Patients were classified as having Fanconi anemia when more than 90% of the cells showed increased breakage. Fanconi anemia mosaicism was defined when there were two populations seen, one with increased sensitivity to DNA cross-linking agents and the other with normal sensitivity to DNA cross-linking agents.

Statistical analysis

Collected data was entered and analyzed using Statistical Package SPSS version 24 (IBM Corp., Armonk, NY, USA). Quantitative (numerical) variables were presented as mean, standard deviation, median, maximum, and minimum) while qualitative (categorical) variables were summarized as frequency and percentage.

Results

Characteristics of study population

Between August 2014 and May 2020, a total of 868 suspected Fanconi anemia patients were referred to Department of Haematology and Cytogenetic, Armed Forces Institute of Pathology, Rawalpindi. One hundred and twenty four (14.29%) were diagnosed as having Fanconi anemia (FA) on chromosomal breakage test. Six of these patients exhibited mosaicism and were classified as FA mosaic.

Of the 124 Fanconi anemia patients, 81 (65.3%) were males while 43 (34.7%) were females with a male to female ratio of 1.9:1. The age of patients ranged from 1 year 06 months to 32 years. Median age of our patients was 09 years 06 months. Consanguinity was observed in 79 (63.7%) and 15 (12%) had a positive family history.

Clinical presentations

Clinical presentations included pallor in 112 (90.3%) patients, bruising and/or bleeding in 34 (27.4%) patients and fever and/or infections in 21 (16.9%) patients. Clinical and radiological examination was done to assess somatic abnormalities. Somatic abnormalities were

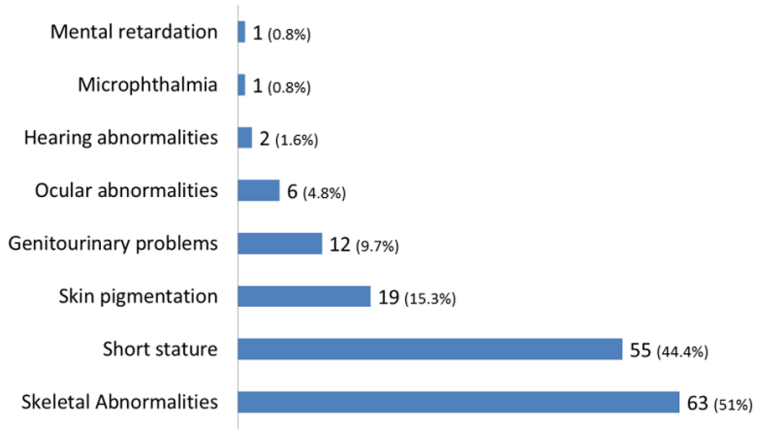

Figure 1. Somatic abnormalities in our cohort of patients.

Table 1. Median haematologic parameters of study population

	Median	Range
WBC ($\times 10^9$/l)	4.8	0.7-8.1
ANC ($\times 10^9$/l)	1.9	0.1-5.1
Haemoglobin (g/dl)	7.1	4.8-11.2
Platelets ($\times 10^9$/l)	88	51-210
Reticulocytes (%)	0.5	0.1-2

Table 2. Bone marrow cellularity of our patients

Bone Marrow Cellularity	n=124	%
<5%	11	8.9
5-9%	29	23.4
10-25%	61	49.2
26-40%	19	15.3
Normocellular	4	3.2

detected in 74 (59.7%) patients while fifty (40.3%) patients had no somatic abnormality. The most common abnormalities observed were skeletal abnormalities and short stature as shown in **Figure 1**. The commonly observed skeletal abnormalities included absent thumbs and radial hypoplasia.

Haematologic parameters

Complete blood counts revealed isolated anemia in 25 (20.2%), isolated thrombocytopenia in 6 (4.8%) and isolated neutropenia in 3 (2.4%) patients. Fifty eight patients (46.8%) were identified to have bicytopenia. Among these, 49 (39.5%) had anemia and thrombocytopenia, 6 (4.8%) had anemia and neutropenia while only 3 (2.4%) had neutropenia and thrombocytopenia. Pancytopenia was observed in 32 (25.8%) patients. Reticulocytopenia was observed in 98 (79%) patients. Median values of different hematological parameters are given in **Table 1**.

Bone marrow examination was done to assess bone marrow cellularity. Bone marrow cellularity of our cohort is exhibited in **Table 2**. Eleven (8.9%) patients had less than 5% bone marrow cellularity while 4 (3.2%) had normal cellular marrows.

Chromosomal breakage analysis

MMC induced chromosomal and chromatid aberrations were observed in 124 Fanconi anemia patients. Eight (6.5%) of these patients also showed spontaneous breaks. Of 124 positive patients, six (4.8%) patients exhibited mosaicism. All controls were negative for spontaneous breaks. However, 2 controls showed positivity for stress-induced breakage. Fifty one (41.1%) patients showed ≥8 breaks/cell while 26 (21%) patients showed ≤5 breaks/cell. The most common chromosomal and chromatid abnormalities observed in our group of patients included chromatid exchanges (triradial, quadriradial) followed by chromatid gaps and breaks, chromosome gaps, breaks and exchanges as well as minutes (as shown in **Figure 2**). Acentric fragments and ring chromosomes were rarely seen.

Discussion

Our study identified 124 (14.29%) patients as having Fanconi anemia. Chowdhry M has reported a frequency of 13.1% in a study conducted in 528 patients [21]. A study conducted in the Iranian population has identified 25% of their study population positive for Fanconi anemia. Talmoudi F has reported a frequency of 19% in the Tunisian population [22]. Data from Italy recorded 94 (52%) males and 86 (48%) females [23]. However, a male predominance was seen in our population and this is most likely due to the fact that our society is male

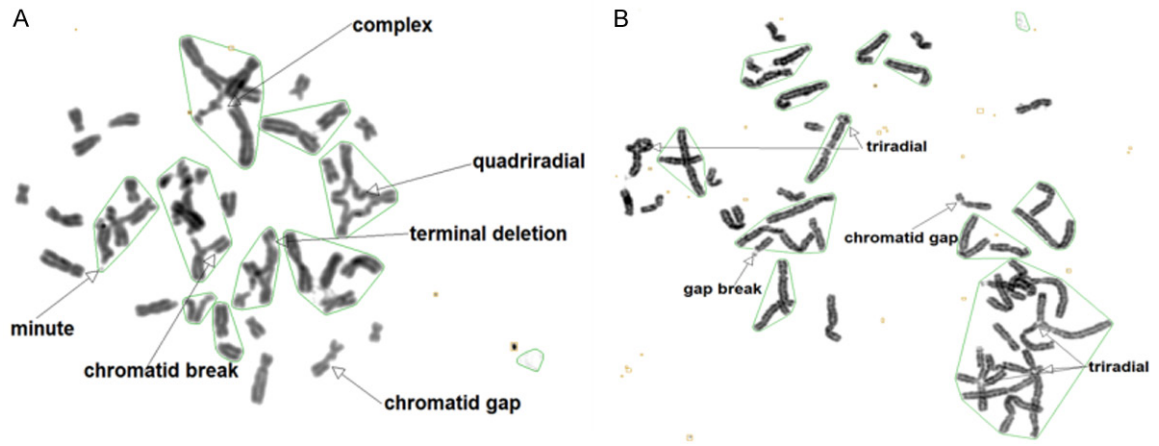

Figure 2. Metaphases of two of the patients showing chromosomal and chromatid abnormalities.

dominated and priority is given to treatment of male children.

In our study, patients presented at a median age of 09 years 06 months (range of 1 year 6 months to 32 years). Antonio M Risitano has observed the median age of Fanconi anemia patients as 7.48 years and an even earlier age of diagnosis in patients born in recent years [23]. A higher age of diagnosis in our patients is probably attributable to the low index of clinical suspicion as frequency of the disease in our population has not been established and also in consideration that being a developing country diagnostic facilities are limited.

Among the 124 Fanconi anemia patients, somatic abnormalities were detected in 74 (59.7%) while fifty (40.3%) patients had no somatic abnormality. The most common abnormalities observed were skeletal abnormalities and short stature. A high frequency (about 75%) of endocrine abnormalities in FA patients, including short stature and/or growth hormone deficiency, hypothyroidism, midline brain abnormalities, abnormal glucose/insulin metabolism, obesity, dyslipidemia, and metabolic syndrome have been mentioned by Shimamura A [24]. In our study, all patients had cytopenias. This is in accordance with cytopenia reported in 97% of the patients by Svahn J in the Italian population [25].

Conclusion

Limitations of this study include lack of molecular diagnosis and the absence of clinical follow-up. Considering ethnic and geographical variability, further studies to determine which Fanconi genes are mutated as well as response of our patients to treatment will be useful for formulating our own clinical practice guidelines. Our study has, however, enhanced the knowledge of this disorder and the clinico-hematologic features in our population, thus, guiding clinicians in early diagnosis and enabling them to institute appropriate management for these patients, thereby, preventing them from DNA cross-linking agents and actively controlling any complications of somatic anomalies, consequently, paving the way to better patient outcome.

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Disclosure of conflict of interest

None.

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