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Clinico-Hematological Characterization of Pakistani Patients with Fanconi Anemia

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Abstract

Objective: This study was designed to characterize the clinical and hematological aspects of Pakistani Fanconi anemia (FA) patients.

Methodology: The study was conducted in Armed forces institute of Pathology Rawalpindi and University of Health Sciences Lahore from 2012 to 2016. Seventy unrelated patients with bone marrow failure were included in this study. Forty FA patients diagnosed by chromosomal breakage analysis were assessed for their demographic, clinical and laboratory parameters. SPSS version 20 was used for statistical analysis.

Results: This study showed mean age of seeking medical aid as 11.21 ±4.22 years. Prenatal consanguinity was 65% and 16 out of 40 patients had siblings affected with FA. All of them had history of repeated infections. There was pleomorphic presentation and varying degree of pancytopenia. Short stature, microcephaly, microphthalmia and café ul lait spots were the common findings.

Conclusion: Fanconi anemia is genetically a heterogenous disease with wide variability of phenotypic presentation. Knowledge of pleomorphic clinical presentation may support health professionals in early diagnosis, evaluation of prognosis, monitoring and genetic counselling of patients and families with Fanconi anemia.

Key words: Fanconi anemia, Inherited, autosomal recessive, Bone marrow failure, Pakistani Consanguinity.

Introduction

Fanconi anemia (FA) is a rare genetically heterogenous disease that was first described by a Swiss Pediatrician Guido Fanconi in 1927. Mode of inheritance in most of the cases is autosomal recessive whereas rarely it is inherited in X-linked pattern (FANCB) and autosomal dominant (FANCR) manner of inheritance has also been described.[1]

The incidence of FA is 1 in 160,000 to 360,000 live births. Though the carrier frequency in general population is 1:300, it is higher in certain ethnic groups such as South African Afrikaners, Spanish Gypsies and Blacks of Sub Saharan. Male and females are equally affected with clinical presentation of bone marrow failure manifestations at median age of 8 years.[2]

Fanconi anemia is characterized by Congenital anomalies, progressive bone marrow failure and early onset malignancies. The most common physical abnormalities include short stature, hyper or hypo pigmentation of skin, Café-au-lait spots, radial ray abnormalities, central nervous system, gastrointestinal, cardiovascular and renal malformations. Progressive bone marrow failure reveals mostly in first decade of life with thrombocytopenia, leukopenia and anemia. Macrocytosis may be the first detected hematological abnormality. Along with these FA patients are predisposed to Acute myeloid leukemia and squamous cell carcinoma.[3,4]

Maintenance of genomic stability depends on specialized mechanisms in the body that serve to repair DNA damage, caused by exogenous or endogenous sources. Hypersensitivity to Interstrand cross linking agents (cisplatin and mitomycin C etc.) and inability of interstrand crosslinks (ICLs) repair is the hallmark feature of Fanconi anemia diagnosis.[5]

Twenty-two FA genes have been identified till date. They include FANCA, FANCB, FANCC, FANCE, FANCF, FANCG (XRCC9), FANCL, FANCM, FANCT (UBET2), FANCP (SLX4), FANCD2, FANCI, FANCD1

Authorship Contribution: [1]Conceived and planned the idea of the study, final approval of the version to be published, Collecting the data, [2]drafting the work or revising it critically for important intellectual content, [3,4]Data Analysis, literature review.

Funding Source: none
Conflict of Interest: none
Received: July 31, 2020
Accepted: Jan 08, 2021

(BRCA2), FANCJ(BRIP1), FANCN (PALB2), FANCO (RADS1C), FANCR (RADS1), FANCS (BRCA1), FANCU(FRCC2), FANCV (REV7/MAD2L2), FANCW (RFWD3) and FANCQ (ERCC4, XPF). The FANC proteins along with associated proteins repair the interstrand crosslinks (ICLs) formed during S phase of the cell cycle by a pathway known as FA pathway of FA/BRCA pathway. Mutation in any of the FA protein impairs the repair mechanism leading to development of disease.[6]

Fanconi anemia is the most common inherited bone marrow failure syndrome. Limited studies and research subjected this disease disavow particularly its prevalence in highly intermingled and consanguineous cultural back ground of Pakistan. In a study conducted by wali et al in 2011 revealed the frequency of FA as 16.6% among 90 aplastic anemia patients.[7] This signifies the importance of FA registry in Pakistan; early diagnosis, timely management and avoidance of complications. FA is phenotypically a heterogenous disease with variable severity. Current study presents the demographic, phenotypic and laboratory parameters of Pakistani FA patients.

Methodology

The study was approved by institutional review committee University of Health Sciences Lahore. Declaration of Helsinki Written, informed consent was obtained from all patients or parents. Study was conducted in n Armed forces institute of Pathology Rawalpindi and University of Health Sciences Lahore.

In this study, we initially included 70 patients with Bone marrow failure from 2012 to 2016 who presented in Armed Forces Institute of pathology Rawalpindi and Children Hospital Lahore. Recruitment of patients was based on detailed history, general Physical and systemic examination and hematological findings of bone marrow failure (BMF). BMF was considered as decrease in one or more blood cell lines in complete blood count, reduced hematopoiesis seen in bone marrow aspiration smears attained with Giemsa stain and hematoxylin-eosin-stained bone marrow trephine biopsies according to the World health organization classification of tumors of hematopoietic and lymphoid tissues 2008. Data was collected and noted in designed proforma. Final diagnosis of Fanconi anemia in these patients was made by positive chromosomal breakage assay i.e., Mitomycin C (MMC) test.

Chromosomal breakage assay

DEB/MMC test was performed according to the protocol described earlier.[8] It was performed by inoculating peripheral venous blood in RPMI 1640 and Fetal bovine serum (FBS) and phytohemagglutinin (PHA). Cultures were kept in Oxygen incubator for 72 hours at 37^{o}C. After 24 hours of inoculation, cultures were induced with Mitomycin C. For each culture a replicate tube was paired as untreated control. Colchicine was added to arrest the cultures at metaphase stage and then treated with Potassium chloride solution (10%). The cells were prefixed with methanol glacial acetic acid solution. Slides were stained with Giemsa stain. Twenty to twenty-five metaphases on at least 4 slides for each patient were seen and scored under bright field microscope and compared with negative controls.

Forty patients with positive DEB/MMC test were recruited after confirmed diagnosis of Fanconi anemia. Patients of any age and both genders were assessed for their demographic, clinical and laboratory parameters.

Results

In this study 40 patients of Fanconi anemia (19M:21F) were included after informed consent. Mean age of patients was 11.21 ±4.22 years ranging from 5 years to 20 years. Weight and height were 10-60 kg (mean 28.00±13.07 kg) and 35-63 inches (mean 47.65±7.18 inches) respectively. Parents of 26 (65%) patients had consanguineous marriage whereas of 14 (35%) patients had no history of consanguinity and were totally unrelated before marriage (Figure 1).

The siblings of 16 patients had the same disease. Bruises or epistaxis found to be the first symptoms and majority of patients seeked medical attention due to recurrent infections with mean age at the time of presentations as 7.95 ± 4.56 years ranging from the age of one month to 19 years. The most common finding in these patients was recurrent infections (100%) followed by pallor (90%), Bruises (72.5%) and epistaxis (52.5%) Thirty-three (82.5%) patients had one or more congenital anomalies whereas 7 (17.5%) patients had no congenital anomaly (Table I)

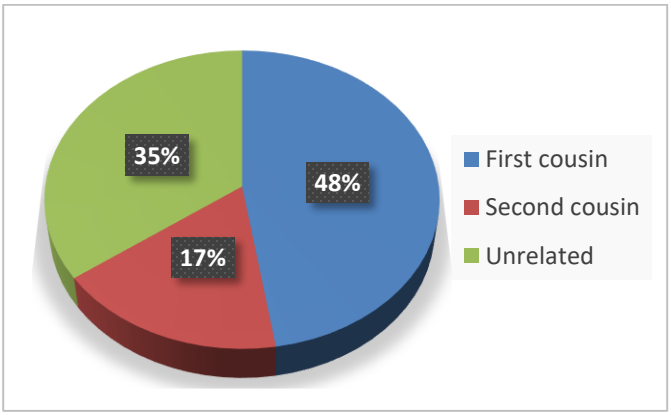

Figure-1: Frequency of prenatal consanguinity among Pakistani Fanconi anemia Patients

Table I: Clinical characteristics of Fanconi anemia patients

Variable		Frequency	%
Gender	**Male**	**19**	**47.5**
	Female	**21**	**52.5**
Consanguinity		26	65
Siblings affected		16	40
Pallor		36	90
Bruises		29	72.5
Epistaxis		21	52.5
Recurrent infections		40	100
Low weight		32	80
Short stature		31	77.5
Microcephaly		25	62.5
Microphthalmia		25	62.5
Café ul lait spots		11	27.5
Thumb abnormalities		12	30
Absent/ectopic kidney		4	10
Cardiac abnormalities		3	7.5
Genital tract abnormalities		2	5

Hematological findings of these patients revealed variable degree of cytopenia with mean Hb 8.04±2.57 g/dl, mean White blood cells 7.40± 27.21 x10^9/L and mean platelet count 57.66±43.26 x10^9/L. All the patients had hypocellular marrow for age. All the patients had normal serum electrolytes and 3 out of 40 FA patients had raised liver enzymes. Figure 2 shows the physical abnormalities in patients of our study.

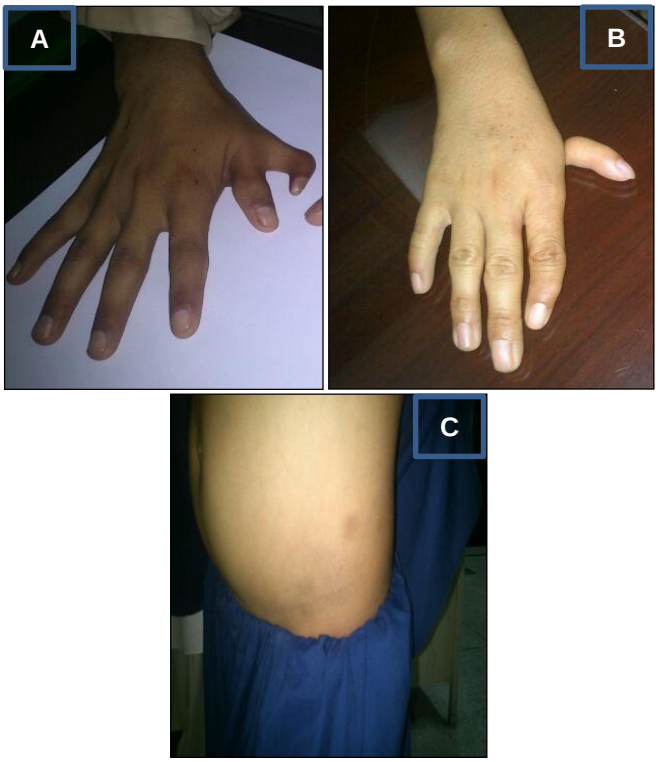

Figure-1: Physical abnormalities in patients with Fanconi anemia of this study. A) Bifid thumb, B) Rudimentary Thumb, C) Café ul lait spots

Discussion

Fanconi anemia is genotypically and phenotypically a pleomorphic inherited disease. It affects all ethnic groups with frequency of 1:360,000 births.[9] Progressive bone marrow failure, high risk of malignancies at an early age and developmental abnormalities are characteristic features. Susceptibility to spontaneous chromosomal breakage is the hall mark of FA cells and chromosomal breakage analysis is the gold standard diagnostic test. Fanconi anemia proteins play a pivotal role in DNA repair mechanism with their associated proteins. Disruption in this repair mechanism by mutated FA proteins leads to Fanconi anemia.[10]

In this study we present a cohort of 40 Fanconi anemia patients. Most of them belonged to Punjab 67.5% (N=27) and rest from Khyber Pakhtunkhwa 32.5% (N=13). High degree of consanguinity was observed. A study conducted in Israel showed 63% consanguinity resulting in this autosomal recessive disorder whereas consanguineous marriages at high level of 94.2 % were responsible for this disorder in a study conducted in Karachi Pakistan.[11,12] Our study showed that 65% patients were offspring of

consanguineous marriages. The results indicate that incidence of Fanconi anemia is higher in couples with family/cousin marriages. Similar results were seen in a Turkish study.[13]

Fanconi anemia is not only a complex disease, but its clinical heterogeneity and overlapping of clinical features with other diseases make it difficult to be diagnosed. Though the disease is associated with the presence of congenital abnormalities, many patients do not have any such abnormalities at the time of birth.[14] Previously, a study conducted by Chowdhry et al revealed common clinical features in 65.8 % of patients, whereas in 34.20% patients there were no apparent symptoms.[15] The most common clinical findings in our patients include short stature, microcephaly, café au lait spots and thumb abnormalities. All patients with congenital anomalies account for 82.55 of those found to have at least one abnormality, 17.5% did not have apparent skeletal deformities. In a previous study done in Mumbai India short stature 81.1 % (N=27) was the most common finding among FA Patients followed by Skeletal abnormalities and skin pigmentation 48.5% (N=16) and 45.5% (N=15) respectively.[16] In our study short stature was the most common finding, and out of 40 FA patients, 77.5% had short stature, followed by microcephaly and microphthalmia each 625% and skin pigmentation in 275% patients. The clinical presentation varies from patient to patient and not all the FA patients have congenital anomalies which signifies the importance of chromosomal breakage analysis in all patients with aplastic anemia for intime diagnosis and early management and diseases associated complications may be avoided.

According to the literature the hematological abnormalities appear at the median age of 7 years.[17] The median age at the time of presentation in our study is 8 years with mean duration of illness as 3.8 years and thrombocytopenia being the first hematological abnormality with spontaneous bruises and epistaxis followed by anemia and finally pancytopenia.

Conclusion

The present study provides the clinical, hematological and biochemical findings in Pakistani Fanconi anemia patients. Our data provided wide phenotypic presentation of FA patients. This heterogenous and multisystemic presentation can offer suspicion to clinicians for timely testing and early diagnosis.

Acknowledgement

We are grateful to patients and families for their participation and willingness to share the findings in scientific publication. Special gratitude to Dr. Shahida Mohsin who perceived this research idea and facilitated at each step. We also acknowledge the cooperation of Armed Forces Institute of Pathology, Department of Hematology, Human Genetics& Molecular Biology, Department of Immunology University of Health Sciences Lahore.

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