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Evaluation of Prevalence and Characteristics of Patients with Fanconi Anemia: A Study in Northeast of Iran

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ABSTRACT

Background and objectives: Fanconi anemia (FA) is an autosomal recessive disorder that usually manifest in forms of pancytopenia, hyperpigmentation, and skeletal complications. Mutation in the DNA repair regulatory genes is associated with the development of FA. Examination of chromosomal breakages when chromosomes are exposed to cross-linking agents is a common method of FA diagnosis. This study aimed to evaluate the prevalence and characteristics of patients with FA in Mashhad, northeast of Iran.

Methods: In this study, we evaluated 312 suspected FA patients who had been referred to the laboratory of Ghaem Hospital during 2014-2020. The mitomycin C method was used to identify FA-positive subjects.

Results: After the examinations, 84 patients (26.9%) were cytogenetically positive for FA. Of 84 patients, 48 (57.1%) were male and 36 (42.9%) were female. Thumb abnormality was the most common congenital anomaly (43.2%).

Conclusion: Based on the findings, males are more susceptible to FA, and thumb abnormality is the most common congenital anomaly associated with FA. Combination of clinical manifestations and genetic susceptibility in patients may contribute to a more accurate diagnosis.

Keywords: Fanconi anemia, Mitomycin, Chromosomal breakages, Congenital abnormality.

INTRODUCTION

Fanconi anemia (FA) is a rare genetic disease caused by an autosomal recessive inheritance, although it can be rarely inherited via X-linked recessive mode (1). This disease affects approximately 1 in 136,000 infants (2). Clinical manifestations of FA include pancytopenia, hyperpigmentation, skeletal abnormalities, small stature, genitourinary abnormalities, and supernumerary thumbs (3). Bone marrow failure is the most common manifestation of FA. Thrombocytopenia with platelet count of more than 30×10^9/l can be often tolerated for years. In addition, hypoplastic hematopoiesis in FA patients can respond to low-dose androgens (4). Researchers have identified genetic mutations in 22 specific FA genes (including FANCA, B, C, D1, D2, E, F, G, I, J, L, M, N, O, P, Q, R, S, T, U, V, W) (5). More than 80% of these mutations occur in the FANCA, FANCG, and FANCC genes; other mutations are rare (6).

The incidence of acute myelogenous leukemia in FA patient is 700-fold higher than in the general population. In addition, FA cases who have received bone marrow transplantation are susceptible to head and neck, esophageal, gastrointestinal, vulvar, and anal cancers. In general, the incidence of malignancy is approximately 50-fold higher in these patients than in the normal population (7). Various approaches including administration of androgen and hematopoietic growth factors as well as hematopoietic stem cell transplantation are currently utilized for treatment of FA (8). Variability of FA phenotypes has limited the diagnosis only to clinical symptoms. Chromosomal fractures occur, when chromosomes are exposed to crosslinking agents, such as diepoxybutane, mitomycin C (MMC), and cisplatin. Examination of such fractures can be used for diagnosis of FA (9). This study is the first to report the prevalence as well as clinical and laboratory characteristics of FA in Mashhad, northeast of Iran.

MATERIALS AND METHODS

We studied 312 suspected FA patients who had been referred to the laboratory of Ghaem Hospital (Mashhad, Iran) during 2014-2020. In this study, we used the MMC method to identify FA-positive subjects. Here, we described a laboratory protocol that has evolved during 20 years of experience. It is recommended for the unambiguous diagnosis of the vast majority of FA patients, including patients with hematopoietic mosaicism. The test is based on the 72 hour-whole blood culture, which is routinely applied in cytogenetic laboratories for karyotyping during which, peripheral blood is cultured in complete RPMI medium with phytohemagglutinin. First, a stock solution of 1.5 mM MMC (0.5 mg/ml) was prepared by adding 4 ml sterile water per vial.

The solution is stable for 3 months at 4 °C. Whole blood culture was performed for all patients (11).

For evaluating FA, peripheral lymphocyte culture was done as follows: three cultures for patients and one for healthy controls. Healthy controls were not siblings of the patients. The cultures were initiated by adding 0.5 ml blood to 4.5 ml complete RPMI medium.

The harvest (incubation, adding hypotonic solution, and washing by fixative) was done at 72 hours, after colcemid treatment. Four microscope slides were prepared for every culture.

Then, the slides were stained by Giemsa and later analyzed for microscopically visible breakage or chromatid-type aberrations (12).

RESULTS

Of 312 suspected FA patients, 84 (26.9%) were cytogenetically positive for FA. The median age of diagnosis was 10 years.

Among 84 FA positive patients, 48 patients (57.1%) were male and 36 (42.9%) were female. The hematological parameters of FA patients are summarized in table 1.

Table1-Hematological parameters of FA patients

Parameter	Mean ± standard deviation
RBC (10^6/µl)	3.80 ± 1
WBC (10^3/ µl)	4.11 ± 1.3
PLT (10^3/µl)	51 ± 16.6
Hb (g/dl)	10.6 ± 2.3
HCT (%)	30.2 ± 6.3
MCV (Fl)	101.2 ± 10.6
MCH (pg)	35.3 ± 4.1
MCHC (g/dl)	34.9 ± 1.1
Neutrophil (%)	22.1 ± 2.4
Lymphocyte (%)	73.4 ± 7.6

RBC: red blood cell, WBC: white blood cells, PLT: platelet, Hb: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

In terms of congenital anomalies, thumb abnormality, short stature, and skin hyperpigmentation were observed in 43.2%, 35.8%, and 32.1% of the studied individuals, respectively. Moreover, all mentioned anomalies were more prevalent in males than in females (Table 2).

Table 2- Prevalence of congenital anomalies in patients with FA

Manifestations	Thumb abnormality	Short stature	Skin hyperpigmentation
Males	46.6%	26.6%	33.33%
Females	37.8%	6.66%	32.4%
Total	43.2%	35.8%	32.1%

DISCUSSION

Fanconi anemia is caused by a defect in a group of proteins involved in DNA repair. It is characterized by progressive bone marrow failure, congenital malformations, and predisposition to malignancy (13). In our study, out of 312 suspected individuals, 84 patients (26.9%) were positive for FA. The number of male patients was slightly higher than female patients (male/female ratio: 1.3). In a previous study in Iran, the prevalence of FA was 19.2%, while females were slightly more affected than males (14), which is inconsistent with our findings. Similarly, in a study in northwestern Iran, Afshar et al. reported the prevalence of FA as 59.4% in females and 40.6% in males (7). However, in line with our findings, some studies also reported that FA was more prevalent in males (15, 16). The inconsistency of findings of previous studies could be related to differences in the cultural and religious beliefs of the studied populations regarding visiting a physician.

While FA often has an autosomal recessive inheritance, it can be rarely inherited via X-linked recessive mode. In this regard, FANCB is the only gene known to be involved in the development of FA via the X-linked inheritance (17). Given the high rate of FA among males in northeast of Iran, there might be an association between the FANCB gene and occurrence of FA in the male population. According to published studies, the age range of FA onset is 5-10 years (median: 7) (18, 19). Less than 4% of FA patients are diagnosed by the age of one, and less than 50% do not have specific hematological manifestations at the time of diagnosis (20). In our study, the age of patients ranged from 5 months to 52 years, and the median age of diagnosis was 10 years.

Complete blood count (CBC) is normal in most FA patients at birth. The most common laboratory findings of patients with FA are thrombocytopenia, anemia, pancytopenia, and macrocytosis (19). In our study, the CBC analysis revealed that most patients had anemia, thrombocytopenia, and macrocytosis. In a study by Tootian et al., the most common laboratory finding among FA patients was anemia (14). Analyzing clinical findings is a critical aspect of FA diagnosis. In this regard, a study reported that most patients diagnosed with FA across Iran had clinical manifestations of the disease (17). The most common clinical manifestations of FA include skin hyperpigmentation, short stature, and abnormal thumb (17). We also found that thumb abnormality, short stature, and skin hyperpigmentation were the most common manifestations of FA. Moghadam et al. reported low birth weight, skin pigmentation, delayed milestone, short stature, and skeletal deformity (thumb) as the most common manifestations of FA (3). Tootian et al. reported thumb abnormality, skin disorders (hypopigmentation), and growth retardation as the most common anomalies associated with FA (14).

The activity of reactive oxygen species (ROS) is not controlled in FA patients. Mitochondria is prone to ROS damage due to insufficient enzymes required for inhibiting ROS, as well as the absence of evolutionary mechanisms in mitochondrial DNA (mtDNA) repair, which can cause cell death and apoptosis. Damage to maternal mtDNA might contribute to progression of disease and symptoms (21, 22). As a result of mtDNA damage due to antioxidant agents, it seems essential to evaluate oxidant status in FA patients.

CONCLUSION

Although FA has several clinical manifestations, CBC could play an important role in confirming hematological disorders in the patients. The results also indicated that FA is more common among males, and thumb abnormality is the most common manifestation of FA among patients in Mashhad, northeast of Iran.

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DECLARATIONS

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Ethics approvals and consent to participate

This study was approved by the Ethics Committee of Mashhad University of Medical Sciences. Patients' data were obtained from the Cancer Molecular Pathology Research Center of Mashhad University of Medical Sciences.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding publication of this article.

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