An Evaluation of the Phenotypic Features of Fanconi Anemia Together with DEB/MMC Positivity in 199 Turkish Patients

Fanconi Anemisinin Fenotipik Özelliklerinin DEB/MMC Pozitifliği ile Birlikte 199 Türk Hastada Değerlendirilmesi

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ABSTRACT Objective: Fanconi anemia is a severe and progressive genetic disease characterized by pancytopenia, a broad range of congenital abnormalities and marked predisposition to malignancy. In this study, the relationship between the clinical manifestations and diepoxybutane/mitomycin C (DEB/MMC) test results was investigated in a group of patients. In particular, the diagnostic significance of minor phenotypic findings was evaluated. **Material and Methods:** A total of 199 cases referred to our department with a diagnosis of Fanconi anemia between 1989 and 2004 were investigated retrospectively. Major and minor clinical findings specific to Fanconi anemia were evaluated in detail. The correlation between chromosomal break ratios occurring either spontaneously or with DEB/MMC induction in lymphocyte cultures from peripheral blood or bone marrow samples and the frequency of phenotypic findings were analyzed with chi-square test and logistic regression. **Results:** Among 199 patients diagnosed with Fanconi anemia, 117 (58.8%) were DEB/MMC (+). Ocular abnormalities, mainly microphtalmia, [31 patients (26.5%) DEB/MMC (+) vs 2 patients (2.5%) DEB/MMC (-)] were the most significant finding followed by café au lait spots [67 patients (57.3%) DEB/MMC (+) vs 15 patients (18.3%) DEB/MMC (-)] and thumb anomalies [52 patients (44.5%) DEB/MMC (+) vs 8 patients (9.8%) DEB/MMC (-)] for the diagnosis of Fanconi anemia. In this study, the two major findings of Fanconi anemia, anemia and growth retardation did not contribute to the logistic regression model for the specific diagnosis of the disease. **Conclusion:** Characteristic findings in Fanconi anemia may lead to diagnosis. However, in certain conditions where classical clinical manifestations are not present it would be particularly important to evaluate the individual malformations, which may be of value for the diagnosis of Fanconi anemia patients during the pre-anemia stage.

Key Words: Fanconi anemia; phenotype; diagnosis; chromosome breakage

ÖZET Amaç: Fanconi anemisi pansitopeni, çeşitli konjenital anomaliler ve maliniteye yatkınlıkla karakterize ağır ve ilerleyici bir genetik hastalıktır. Hastalarda gözlenen klinik bulgular geniş bir yelpazede yer almaktadır. Bu çalışmada Fanconi anemisinin klinik bulguları diepoksibütan/mitomisin C (DEB/MMC) testi sonuçları ile birlikte analiz edilerek, fenotipik özellikler ile DEB/MMC testi sonuçları arasındaki ilişki araştırılmış, özellikle minör olarak tanımlanan fenotipik bulguların tanıdaki önemi değerlendirilmiştir. **Gereç ve Yöntemler:** 1989-2004 yılları arasında bölümümüze Fanconi anemisi ön tanısı ile gönderilen 199 olgu geriye dönük olarak incelenmiştir. Fanconi anemisinde tanımlanan major ve minör fenotipik değişiklikler başta olmak üzere, hastaların fizik muayene bulguları ayrıntılı olarak incelenmiştir. Olguların periferik kan veya kemik iliği lenfosit kültürlerinde spontan ve DEB/MMC ile uyarılmış kromozom kırık oranları ile saptanan fenotipik bulgular birlikte değerlendirilmiştir. DEB/MMC testi sonuçları ile fenotipik değişiklikler arasındaki ilişki lojistik regresyon analizi, fenotipik değişikliklerin sıklığı ve birbirleriyle olan ilişkileri ise ki-kare testi ile incelenmiştir. **Bulgular:** Fanconi anemisi öntanısı ile gelen 199 hastadan 117'sinde (%58,8) DEB/MMC testi pozitif bulunmuştur. Anemi ve gelişme geriliği, Fanconi anemisindeki temel bulgulardan olmakla birlikte, bu bulguların, hastalığın ayırt edici özellikleri açısından lojistik regresyon modellerine etkisinin sınırlı olduğu görülmüştür. Bu çalışmada, mikroftalmi başta olmak üzere göz bulguları [DEB/MMC testi (+) 31 hastada (%26,5), DEB/MMC testi (-) 2 hastada (%2,44)], sütlü kahve lekeleri [DEB/MMC testi (+) 67 hastada (%57,26), DEB/MMC testi (-) 15 hastada (%18,29)] ve başparmak anomalilerinin [DEB/MMC testi (+) 52 hastada (%44,44), DEB/MMC testi (-) 8 hastada (%9,76)] tanıya katkısı açısından istatistiksel anlamlılığı en yüksek bulgular olduğu ortaya konmuştur. **Sonuç:** Hastalığın karakteristik bulguları ön tanı için yol göstericidir. Ancak bu bulguların klasik olarak bir arada bulunmadığı durumlarda tek tek malformasyonların değerlendirilmesi, hastaların, anemi gelişmeden önceki dönemde fark edilmesine katkı sağlayacaktır.

Anahtar Kelimeler: Fankoni anemisi; fenotip; tanı; kromozom kırılması

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Fanconi anemia (FA) was first described in 1927 by Guido Fanconi.[1] It is a rare disease (FA; OMIM#227650), which is mostly inherited autosomal recessively and less frequently in an X-linked pattern.[2] The prevalence of FA is between 1/26,000 and 1/476,000 in different geographic regions.[3,4] Until 1992, approximately 1,000 patients have been reported. In addition to its vast phenotypic variations, FA is genetically heterogeneous with 15 disease-causing genes described.[5,6] The pancytopenia, the growth delay and the hyperpigmentation of the skin, which are among the most constant clinical characteristics of FA are present in over 60% of patients.[7] The most frequently associated malformations are those of the skeletal system, mainly of the radius and thumb. Less than 50% have renal, genital, ocular, hearing or heart abnormalities.[8,9] The presence of malformations such as anal atresia, cardiac defects, tracheoesophageal fistula in FA patients may lead to confusion with other entities such as the VACTERL association or the Baller-Gerold syndrome.[9-11] Approximately 20% of patients develop some type of neoplasia, mainly hematological (leukemias) and carcinomas, particularly liver cancer.[12] Generally, a FA patient has 15,000 times greater risk of developing cancer in pediatric ages.[1,7] Reports suggest that 24% of FA patients develop leukemia as the first symptom of the disease. The most frequent type of leukemia in FA patients is acute myeloid leukemia, which may or may not be preceded by myelodysplastic syndrome.[13] The FA clinical picture is extremely variable. Up to 37% of the patients do not have any associated congenital malformations.[14] In such cases, the diagnosis is not made until the onset of pancytopenia. This hematological manifestation, which is present in 90% of cases, also shows variation in the degree of severity, and the age of onset.[1] Although most of the patients are diagnosed between 3 to 7 years of age based on the presence of pancytopenia, the diagnosis in 10% is made after the age of 16. The range of age at diagnosis varies between 0 and 38 years and the most frequent initial hematological manifestation is thrombocytopenia. The clinical picture has a continuous spectrum of manifestations. Some patients have a relatively mild condition with normal skeletal development and subclinical hematopoietic abnormalities and they can survive to the fourth or fifth decade. At the other end of the scale are patients with severe phenotype, multiple skeletal abnormalities, and an early onset of aplastic anemia and/or cancer. There are also atypical, late onset clinical conditions without anemia, or with initial conditions that may be confused with other syndromes, such as those mentioned earlier.[14,15] The definitive diagnosis of FA is established when the lymphocytes from peripheral blood or bone marrow cultures react to mitomycin C (MMC) or diepoxybutane (DEB) induction by chromosome breaks 3-10 times greater than a normal control. These chromosomal breaks are due to defects originating from FA genes (*FANC*), which are the essential elements of DNA repair mechanism.[6] Comparative studies show that the DEB test is highly sensitive and specific given that the chromosomal response of FA patients cannot be confused with the response of a normal control.[15,16] Although the DEB test is highly effective in discriminating FA from other conditions, it remains underutilized mainly because there are some clinical conditions in which FA is not usually suspected. The purpose of this study was to screen patients for the manifestations related to the FA phenotype together with DEB/MMC test results and to evaluate the presence or absence of individual phenotypes that may serve as diagnostic criteria. This evaluation may help to diagnose the disease especially in its early stages with atypical or minor clinical manifestations and may help to define the conditions that should be considered in routine examinations for the diagnosis of the disease.

MATERIAL AND METHODS

CASES

A total of 199 cases referred from the pediatric and adult hematology clinics to our department with a diagnosis of FA between 1989 and 2004 were included in this study. Patients underwent a routine systematic examination as well as pedigree analyses at the first visit. We re-evaluated the results from patient files and looked specifically for the existence of major and minor clinical criteria. All but 12 cases had anemia and/or malformations at the time of referral. The 12 cases were included in the

study for their family relations with the patients. This study was approved by the Ethical Committee for Clinical Research of Ankara University Faculty of Medicine (1882 /24.01.2011).

DEB/MMC TESTS

Peripheral blood samples or bone marrow samples were obtained for the detection of hypersensitivity to the clastogenic effect of the DNA cross-linking agent DEB in cultures along with untreated cultures for the detection of spontaneous chromosomal breaks according to a standard protocol used in our department. In cases where DEB induced cell cultures did not yield adequate metaphases, MMC was used for induction. Cells were incubated for 48 hours in conventional peripheral blood lymphocyte cultures with the addition of DEB (0.01 μg/ml) and/or MMC (0.01 μg/ml) at the last 24-hour-period. Cultures were harvested, slides were prepared and analyses were performed on 50 Giemsa-stained metaphases, each from induced and uninduced conditions for the quantity and the quality of the breaks. We used a cut-off value of 0.1 and 0.16 chromosome breaks per cell for spontaneous or induced cell cultures respectively. We considered a test positive when the rate of the chromosome breaks from DEB induced cultures was twice the rate of the spontaneous breaks.[17]

STATISTICAL ANALYSES

Clinical variables and DEB/MMC induced chromosomal break results were evaluated with the criteria described in the previous section. Data were summarized as mean ± standard deviation (SD) for normally distributed quantitative variables. Where normality was not met, median (min.-max.) along with mean ± SD were used as descriptive statistics. Independent group comparisons were analyzed with Mann Whitney U test and Chi-Square test for non-normal quantitative and qualitative variables, respectively. Logistic regression analysis was used to determine the risk factors in FA. First, data from clinical variables were examined with the chi-square test, and then significant factors were included in the binary logistic regression analysis. The logistic regression equation was formulated with a backward LR selection method.[18] All statistical analyses were performed with SPSS 15.0 for Microsoft Windows (SPSS, Chicago, IL).

RESULTS

The mean age and standard deviation of the 82 female and 117 male patients at diagnosis were 8.83±4.9 with a median of 8 (1-32). The results of the clinical findings of the patients were listed in Table 1 and 2 in 5 age groups.

TABLE 1: The clinical findings of DEB/MMC (+) cases.

	0-3		4-6		7-9		10-12		13-older	
Age at diagnosis	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Gender(male-female)	3-6	33-67	16-20	44-56	10-21	33-67	9-12	43-57	10-10	50-50
Consanguinity	6	66.67	26	72.2	24	77.4	19	90.48	16	80.00
Family history	1	11.11	6	16.7	7	22.6	3	14.29	4	20.00
Anemia	5	55.56	26	74.3	25	80.6	17	80.95	17	85.00
Ear anomaly	1	11.11	6	16.7	3	9.7	1	4.76	2	10.00
Ocular anomaly	2	22.22	7	19.4	12	38.7	6	28.57	4	20.00
Growth retardation	3	33.33	22	61.1	12	38.7	9	42.86	8	40.00
Thumb anomaly	6	66.67	18	50.0	14	45.2	8	38.10	6	30.00
Café au lait spots	3	33	23	63.9	21	67.7	13	61.90	7	35.00
Other pigmentation anomalies	0	0.00	15	41.7	9	29.0	8	38.10	5	25.00
Renal anomaly	1	11.11	9	25.0	3	9.7	0	0.00	2	10.00
Skeletal anomaly	1	11.11	2	5.6	3	9.7	0	0.00	1	5.00
Urogenital anomaly	4	44.44	6	16.7	2	6.5	4	19.05	2	10.00
Lower extremity anomaly	2	22.22	6	16.7	6	19.4	1	4.76	3	15.00
Other upper extremity anomaly	6	66.67	22	61.1	18	58.1	13	61.90	7	35.00

TABLE 2: The clinical findings of DEB/MMC (-) cases.

	0-3		4-6		7-9		10-12		13-older	
Age at diagnosis	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Gender (male-female)	3 -11	21-79	9-7	56-44	5-6	45-55	10-4	71-29	7-20	26-74
Consanguinity	5	64.3	11	68.75	6	54.55	5	35.71	11	40.74
Family history	0	0	1	6.25	0	0.00	1	7.14	9	33.33
Anemia	6	64.3	13	81.25	8	72.73	8	57.14	15	55.56
Ear anomaly	0	0	1	6.25	1	9.09	1	7.14	2	7.41
Ocular anomaly	1	14.3	0	0.00	0	0.00	0	0.00	0	0.00
Growth retardation	3	50	5	31.25	3	27.27	3	21.43	5	18.52
Thumb anomaly	3	21.4	3	18.75	0	0.00	0	0.00	2	7.41
Café au lait spots	2	21.4	2	12.50	2	18.18	1	7.14	7	25.93
Other pigmentation anomalies	1	14.3	1	6.67	1	9.09	1	7.14	3	11.11
Renal anomaly	0	0	0	0.00	0	0.00	1	7.14	1	3.70
Skeletal anomaly	0	14.3	1	6.25	2	18.18	2	14.29	4	14.81
Urogenital anomaly	0	7.14	1	6.25	0	0.00	1	7.14	0	0.00
Lower extremity anomaly	3	28.6	1	6.25	1	9.09	3	21.43	1	3.70
Other upper extremity anomaly	5	42.9	4	25.00	2	18.18	3	21.43	5	18.52

Of 199 cases, 179 were tested for DEB induced chromosomal breaks. Sixteen cases were also tested for MMC induction for reasons such as strong clinical evidence of FA or where DEB induced cultures failed to provide the required number of metaphases. The remaining 20 cases were only tested with MMC induction. The means for chromosomal breaks and method of inductions were given in Table 3. The clinical features of patients were evaluated in two categories as DEB/MMC (+) and DEB/MMC (-). Results showed that DEB/MMC (+) was not related to gender (for positive males n=69 (59%), for positive females n= 48(41%); for negatives males n=48 (58.5%), for negatives females n=34 (41.5%); p=0.951). These two groups did not show a significant difference regarding their ages; 8.56±4.7_with a median of 8 (1-32) and 9.21 ±5.1 with a median of 9.5 (1-21) respectively in patients with DEB/MMC (+) versus DEB/MMC (-) results were (p=0.26). Consanguinity between parents in DEB/MMC (+) and (-) cases was 77.7% and 51.2% respectively (p<0.001).

The frequency of the clinical findings in patients with DEB/MMC (+) and DEB/MMC (-) results were summarized in Table 4. A bivariate, stepwise logistic regression analysis was carried out using a list of independent variables (Table 5). Anemia and growth retardation are two central findings for FA. However, they did not contribute to the logistic model for depicting FA in our study (Table 4). The frequency of anemia was very high in both groups. The frequency of patients with cafe au lait spots or hypo/hyperpigmentation was three times higher in DEB/MMC (+) cases compared to DEB/MMC (-) patients. Microphtalmia, strabismus, deep-set eyes and anomalies of the upper eyelid were grouped as ocular anomalies. Microphtalmia was detected in approximately half (16) of the patients who had ocular anomalies. In all cases, microphtalmia was accompanied with microcephaly. However, ocular anomalies as a risk factor revealed the highest odds ratio followed by café au lait spots and thumb anomalies for FA di-

TABLE 3: Mean chromosome breaks/cell in DEB/MMC (+) and DEB/MMC (-) patients.

	% in DEB/MMC (+) (±SD)	% in DEB/MMC (-) (±SD)
Condition	**(n) patients**	**(n) patients**
Spontaneous	21.38 (±16.44) (117)	2.02 (±2.62) (82)
DEB Induced	89.95 (±86.07) (105)	3.82 (±7.65) (74)
MMC Induced	168.94 (±186.54) (18)	6.00 (±4.66) (18)

DEB: Diepoxybutane; MMC: Mitomycin C.

TABLE 4: Comparison of DEB/MMC (+) vs. DEB/MMC (-) clinical variables.

	DEB/MMC (+)		DEB/MMC (-)		
Clinical Variables	**n =117**	**%**	**n =82**	**%**	**p**
Anemia	90	76.92	53	64.63	0.058
Cafe au lait spots	67	57.26	15	18.29	<0.001
Growth retardation	54	46.15	23	28.05	0.010
Thumb anomalies	52	44.44	8	9.76	<0.001
Hypo-hyperpigmentation	37	31.62	8	9.76	<0.001
Ocular anomalies	31	26.50	2	2.44	<0.001
Genital anomalies	18	15.38	3	3.66	0.008
Lower extremity anomalies	19	16.24	10	12.2	0.426
Renal anomalies	15	12.82	2	2.44	0.010
Ear anomalies /hearing loss	13	11.11	5	6.10	0.225
Skeletal anomalies	7	5.98	11	13.41	0.072

n: total number of patients; p: probabilities by chi-square test; DEB: Diepoxybutane; MMC: Mitomycin C.

agnosis (Table 5). Hypogonadism, which is considered to be a rare anomaly in FA, was detected more frequently in DEB/MMC (+) patients. The renal anomalies of the patients in this study consisted of unilateral renal agenesis and hypoplastic, horseshoe, rotated or ectopic kidney. There was also a significant difference between the two groups for the presence or absence of renal anomalies favoring DEB/MMC (+) cases. Our observations revealed that outer ear anomalies were the most common recurrent finding in patients with ear anomalies. Deafness, abnormally positioned ear, small or absent ear canal were also present in a number of patients. Although the difference between DEB/MMC (+) and DEB/MMC (-) groups for ear abnormalities was not as high as the renal abnormality, it was close to two-fold (Table 4). The eleven independent variables obtained from the clinical data of DEB/MMC (+) and (-) cases were first analyzed with the chi-square test. Four of the eleven variables were eliminated regarding the *p* values the tests yielded. Since pigmentation abnormalities and café au lait spots have casual relations, the former was also excluded from further analysis. The remaining six variables were used to build the logistic regression model by backward stepwise likelihood ratio selection method. A five-variable model, namely including ocular abnormalities, café au lait spots, thumb anomalies, consanguinity, and skeletal abnormalities, was selected as the final model, which discriminates best between DEB/MMC (+) and (-) cases (Table 5).

TABLE 5: Odds Ratios (OR) from binary logistic regression for DEB/MMC positivity.

		95% confidence interval for OR		
Clinical/Pedigree Parameters	**OR**	**Lower**	**Upper**	**p**
Consanguinity (+ vs -)	4.251	1.807	10.004	0.001
Ocular findings (+ vs -)	25.753	4.129	160.619	0.001
Thumb anomalies (+ vs -)	4.994	1.928	12.937	0.001
Café au lait spots (+ vs -)	6.788	2.839	16.227	<0.001
Skeletal abnormalities (+ vs -)	0.108	0.026	0.454	0.002

Hosmer and Lemeshow goodness of fit test: p=0.704

Omnibus tests of model coefficients p<0.0001; -2 Log likelihood=174.506

DEB: Diepoxybutane; MMC: Mitomycin C.

The sensitivity and the specificity of the assumed model were calculated as 78.6% and 86.6% respectively.

DISCUSSION

FA is a genetically and phenotypically heterogeneous disease. It is mostly characterized by the presence of major and/or minor congenital malformations, pancytopenia and predisposition to malignancies. However, the range of malformations may vary from one to many.[14] Balaban et al. evaluated 21 cases with distinct phenotypic combinations and given the fact that the disease may

present itself with a broad variety of findings, they suggested a special attention from clinical point of view in order not to miss a FA patient in the first encounter.[19] Therefore, when a patient's clinical manifestations comply with the characteristic findings of FA, the physician's decision towards a FA diagnosis is more likely, otherwise a patient with FA can easily be missed. Are there any minor clinical features that could be significant from the diagnostic point of view in FA? To answer this question, phenotypic variables of DEB/MMC(+) and DEB/MMC(-) patients at the time of their referral were investigated. The cases in which the presence of anemia could not be related directly with a disease condition can be candidates for FA diagnosis. The high frequency of anemia in our study group, either DEB/MMC (+) (77%) or (-) (65%), supports this fact. Although anemia alone cannot clearly discriminate FA from other diseases, it ensures that a true instance of FA will not be missed. The ratio of patients with cafe au lait spots or hypo-hyperpigmentation in DEB/MMC (+) group was 57.3% and 31.6% respectively. These figures were significantly lower in DEB/MMC (-) group (18.3%, 9.8% respectively). In 42 FA cases from Turkey Azık et al. reported that 95.2% of the patients had pigmentation abnormalities (cafe au lait, hypo/hyperpigmentation combined).[20] In our series the frequency of the skin findings was 88.9% in FA patients which was lower than that in the study by Azık et al. However, more than three-fold higher presentation of abnormal skin pigmentations in DEB (+) versus DEB (-) cases should still be considered significant ($p<0.001$). The marked differences between those two groups in contrast with anemia may lead the physician to a FA diagnosis.

Table 6 shows that the frequency of DEB/MMC (+) cases that have both anemia and at least one malformation (1st category) was 71.8% and was twice as many as the DEB/MMC (-) cases within the same category (35.4%). There were few cases (n=9) in the DEB/MMC (+) group that did not show any malformations and only three of those patients (4th category) had neither anemia nor any malformations. Therefore, from a clinical perspective, the co-existence of anemia with at least one malformation is worth noticing, as the presence of FA and anemia alone weakens the possibility of FA. The cases in the 3rd and 4th categories that lack anemia may be caused by misdiagnosis. When these 24 cases versus 20 DEB/MMC negatives in the 3rd category were compared at least for some of the clinical manifestations such as cafe au lait spots (14 vs. 5), growth retardation (14 vs. 11) and thumb anomalies (11 vs. 2) there seemed to be differences but it turned out to be statistically significant only for thumb anomalies. Although the difference between these two groups is statistically insignificant, it is still a good indication of how a detailed physical examination may contribute to a correct diagnosis of FA. The distribution of the ages of DEB/MMC (+) patients in the 3rd and 4th categories showed that more than half (n=18) were eight years of age or under, and only three were 13 years and over. Several studies showed that the anemia might appear later in life.[14,21,22] In the absence of anemia, patients with other clinical findings should be noticed by the physician for the FA diagnosis. In FA, approximately two-thirds of the patients have major congenital malformations.[18] It is evident that the presence of any major malformation can facilitate the diagnosis and minor malformations should not be overlooked either.

Microphtalmia and microcephaly were categorized as minor malformations by the International Fanconi Anemia Registry (IFAR).[23] Ocular anomalies, mainly microphtalmia showed the highest OR followed by café au lait spots and

TABLE 6: Frequency of DEB/MMC (+) (117) and (-) (82) patients according to presence of anemia and/or clinical findings (including growth retardation).

Categories	DEB/MMC (+) n (%)	DEB/MMC (-) n (%)	Total (%)
1. Anemia+/malformations +	84 (71.8)	29 (35.4)	113 (56.8)
2. Anemia+/malformations -	6 (5.1)	24 (29.3)	30 (15.1)
3. Anemia-/malformations +	24 (20.5)	20 (24.4)	44 (22.1)
4. Anemia-/malformations -	3 (2.6)	9 (11.0)	12 (6.0)
Total	117 (100)	82 (100)	199 (100)

DEB: Diepoxybutane; MMC: Mitomycin C.

thumb anomalies for FA in this study (Table 5). We saw that all cases with microphtalmia were also microcephalics but not all microcephalics were microphtalmic. This may suggest that the presence of microphtalmia can be critical in FA diagnosis with atypical clinical conditions while microcephalia alone is inadequate for the exclusion of FA.

In our study, ear anomalies were present in 11% of the cases with DEB/MMC (+) test results. This was lower than the percentage in IFAR (21.6%) obtained from a collection of 1075 patients. This may be attributed to the criteria chosen for ear examinations and especially for minor ear anomalies such as small differences in helical structures.[23] One third of our cases who were detected with renal anomalies also had ear anomalies and it was worth noticing that half of the ear findings were minor ones.

Consanguinity among parents in DEB/MMC (+) cases was 77.7% while in the negative cases it was 51.2%. In a FA series of 65 cases from Turkey, the results for consanguinity were comparable to the results in our study.[4] Among patients, this rate was 78% versus 46% in the non-FA group. The demographic studies in Turkey show a high consanguinity rate.[24] The frequency of consanguinity in cases with anemia that were tested positive with DEB/MMC was 73% and suggests a causal relation.

FA is a genetically heterogeneous disease in which 15 different genes have direct causal effects with numerous mutations they bare.[5,6] Recent research activities with regard to FA mainly focus on the genotype-phenotype relations and on the role of FANC proteins in the DNA repair pathway. Some of these studies indicate a relation between mutation type such as *FANCC* (IVS+4A>T) substitution and clinical manifestations in certain ethnic groups.[25] The vast number of mutations prevent mutation testing on the patients from being a part of the routine analysis scheme with the exception of some ethnic groups in which particular mutations can be seen frequently. Null mutations of *FANCA*, or specific mutations in *FANCC* and *FANCG* were more often related to severe clinical types.[26] In addition to mutations in FA genes modifier gene effects and environmental factors are also under investigation.[27] Nevertheless, there is no clear explanation for phenotypic variations and mutation types nor there is an obvious correlation between the symptoms and the extent of sensitivity to DNA cross-linking agents in FA. There are still some FA patients reported with unassigned subtypes, and it is suggested that the identification of additional FA or FA associated genes will give further insights into the molecular functions and mechanism of the FA pathway.[28]

In conclusion, this study revealed that microphthalmia, thumb anomalies and café au lait spots are significantly more frequent in DEB/MMC(+) cases. Anemia alone is not a determinate of FA. The co-existence of anemia and malformations, and the presence of consanguinity in patients' parents increase the probability of FA in the patients. Cases with growth retardation and/or anemia should receive a thorough examination for minor phenotypic features-even ear and kidney anomalies should not be overlooked- in order to prevent an omission in FA diagnosis.

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