Lymphocyte mosaicism in Fanconi anemia: diagnosis and clinical correlation

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Background and aim
Increased chromosome breakage is the diagnostic feature of Fanconi anemia (FA) owing to genomic instability. Patients with hematopoietic somatic mosaicism are often difficult to identify clinically and diagnostically, although they may comprise up to 25% of patients with FA. The study aimed to clarify the variability of chromosomal breakage test in diagnosis of FA and lymphocyte mosaicism in addition to studying the relation between chromosomal breakage test results and clinical data of the patients.

Patients and methods
The study included 25 patients with clinical suspicion of FA. Moreover, 225 age-matched and sex-matched healthy volunteers were included as a control group. Chromosomal breakage test at 300-nM mitomycin C (MMC) concentration was done.

Results
According to the results of chromosomal breakage test at 300-nM MMC concentration, patients were divided into Fanconi and non-Fanconi groups. The mean chromosomal breakage per cell was 4.67 ± 1.58, per aberrant cell was 5.6 ± 1.1, and the mean chromosomal fragility index was 466.7 ± 157 in the Fanconi group, which was significantly different from non-Fanconi group. Patients with FA were further divided into classic Fanconi anemia (CFA) with more than or equal to 10 breaks/cell and Fanconi with mosaicism (FA-Mo) with two cell populations at 300-nM MMC, one like typical FA cells showing more than or equal to 10 breaks/cell, and one like healthy controls, which was largely represented by the categories 0, 1, and 2 breaks/cell. There were significant differences between CFA and FA-Mo regarding results of chromosomal breakage test at 300 nM MMC per cell, per aberrant cell, and the chromosomal fragility index. Insignificant differences were observed between CFA and FA-Mo regarding clinical or hemogram data.

Conclusion
The present study provided a detailed laboratory protocol for the accurate assessment of the FA diagnosis and enabled a quantitative estimate of the degree of mosaicism in the lymphocyte compartment of the patient.

Keywords:
bone marrow failure, chromosomal breakage test, Fanconi anemia, somatic mosaicism

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Introduction

Fanconi anemia (FA) is an inherited syndrome characterized by progressive bone marrow failure (BMF), abnormal skin pigmentation, short stature, and increased risk of cancer. BMF in FA is multifactorial and mostly results from the death of hematopoietic stem cells owing to genomic instability (Shabrish *et al.*, 2019).

It is phenotypically and genetically heterogeneous, where at least 22 genes code for products that cooperate in DNA repair pathway. The inheritance is autosomal recessive, except FA type B, which is X-linked recessive (Bogliolo *et al.*, 2013). So far, 22 FANC genes have been identified, among which the FANCA genes have been reported as the most common (Zareifar *et al.*, 2019).

Biallelic mutations of FANCD2 and other components of the FA pathway cause a disease characterized by BMF, cancer predisposition, and a striking sensitivity to agents that induce cross-links between the two complementary DNA strands (interstrand cross-links-ICL). These genotoxins were used to characterize the contribution of the FA pathway to the genomic stability of cells, thus unraveling the biological relevance of ICL repair in the disease process (Federico *et al.*, 2018).

The FA pathway is inactive in normal cells, whereas it is turned on during the S phase of cell cycle or in the presence of DNA damage proteins, and it also plays an important role in DNA repair pathway as part of cellular defense against DNA interstrand cross-linkers (Kee and D'Andrea, 2010).

Additionally, other DNA repair factors and DNA damage checkpoint proteins such as ATR (ataxia telangiectasia mutated Rad3-related), CHK1 (checkpoint kinase 1), and γ-H2AX (histone complex which is a novel biomarker for DNA double strand breaks) are involved. These proteins interact with the FA pathway in response to DNA damage, leading to cell cycle arrest after DNA damage, thus preserving genomic stability (Moldovan and D'Andrea, 2009).

Recommendations for the diagnosis of FA were agreed upon at a 2008 consensus conference (Eiler *et al.*, 2008). Patients with FA might have physical abnormalities including short stature; abnormal skin pigmentation; malformations of the thumbs, forearms, skeletal system, eye, kidneys and urinary tract, ear, heart, gastrointestinal system, oral cavity, and central nervous system; hearing loss; hypogonadism; developmental delay; progressive BMF; adult-onset aplastic anemia (in which red cell macrocytosis and elevated hemoglobin F levels may be seen); myelodysplastic syndrome or acute myelogenous leukemia; and solid tumors.

The diagnosis of FA is based on cytogenetic testing for increased chromosomal breakage (Auerbach, 1993) or presence of radial figures using diepoxybutane (DEB) or mitomycin C (MMC) (Cervenka *et al.*,1981). Abnormal bone marrow cytogenetic findings may also develop (Cioc *et al.*, 2010). Evaluation of FANCD2 protein monoubiquitination by immunoblotting also acts as a rapid diagnostic test for FA. However, rare FA subtypes FA-D1 (BRCA2), FA-J (BACH1/BRIP1), and FA-N (PALB2) may be missed with this approach (being downstream of FANCD2), and also individuals with somatic mosaicism may also be missed (Shimamura *et al.*, 2002).

Lymphocyte mosaicism occurs in a sizable portion of patients with FA (estimated at 10–30%). The somatic molecular events underlying mosaicism in patients with FA include back mutation, intragenic crossover, gene conversion, or compensating deletions/insertions, leading to correction of one of the mutated FA alleles in either a hematopoietic stem cell or lymphocyte progenitor (Alter *et al.*, 2005).

In most FA cases with mosaicism, diagnosis is done by testing peripheral blood, as some of the cells will still show hypersensitivity to cross-linking agents (Gross *et al.*, 2002). Chromosomal aberrations have been analyzed in many ways, including number of breaks per cell, number of breaks per aberrant cell, and chromosomal fragility index (CFI) (John *et al.*, 2014).

Occasionally, the percentage of reverted cells may reach a high level that may lead to a false-negative diagnosis. In such cases, cross-linker sensitivity may be tested on skin fibroblasts, which are not known to be affected by mosaicism. After a positive breakage test result is obtained, screening for mutations in the known FA genes is warranted (Gross *et al.*, 2002).

Studies were done to correlate the results of chromosomal breakage and clinical data where some clinical records for mosaic cases showed normal or mildly decreased blood cell counts with stability or improvement in the clinical condition (Gross *et al.*, 2002).

The aim of the current study was to clarify the variability of chromosomal breakage test in diagnosis of FA and lymphocyte mosaicism and to study the relation between chromosomal breakage test results and clinical data of the patients.

Patients and methods

A total of 25 patients with clinical suscpicion of FA were included in our study. Patients were selected from the Hematology Clinic, Abo Elreish Hospital, Cairo University. Clinical data of patients with FA were obtained, including duration of the disease and the number of congenital malformations, including skeletal, gastrointestinal, cardiac, and genitourinary system malformations. Informed consents were taken from patients or parents.

A total of 25 age-matched and sex-matched healthy volunteers, not relatives of the patients, were included in the current study as a control group.

Patients were subjected to full history taking, thorough clinical examination, thorough radiological assessment, and laboratory investigations, including complete hemogram and reticulocytic count, bone marrow aspiration and biopsy, and peripheral blood mononuclear cell culture set for chromosomal breakage test with addition of clastogenic agent MMC according to the method described by Oostra *et al.* (2012). Controls were subjected to complete hemogram, reticulocytic count, and chromosomal breakage test.

Ethical approval

The study design was approved by the Scientific Research Committee and ethical committee of the clinical pathology Department, Faculty of Medicine, Cairo University. Data confidentiality was observed according to the Revised Helsinki Declaration of Bioethics.

Cell cultures

Three cultures for each patient and each control were prepared at 0-, 150-, and 300-nM MMC concentrations.

Analysis was performed on 50 Giemsa-stained metaphases; each cell was scored for chromosome number, as well as number and types of structural abnormalities.

Chromosomal aberrations were analyzed, including number of breaks per cell, number of breaks per aberrant cell, and CFI (percent of aberrant cells x breaks per aberrant cell) (John *et al.*, 2014). Aberrations included chromatid gaps, breaks, triradial and quadriradial chromosomes, and other chromatid interchange figures. Chromatid gaps or breaks were counted as single-break events, and triradial and quadriradial as two break events each. Cells showing more than 10 break events are not further quantified and are included in a common category 'more than or equal to 10 breaks/cell.'

Cultures exposed to 0- and 300-nM MMC were first scored where the diagnosis 'FA' was warranted if all metaphases showed multiple aberrations, whereas the majority of the control cells were normal. If too few evaluable metaphases were found or indications of mosaicism were seen, samples exposed to 150 nM were scored.

Cultures from typical patients with FA should show aberrations in the majority of cells at 150-nM MMC, no undamaged cells should be left at 300-nM MMC, and most cells should be in the category 'more than or equal to 10 breaks/cell.'

In contrast, cultures from healthy control should hardly or not be affected, except at 300 Nm, where typically 30% of the cells may show 1 to less than or equal to five break events/cell. In cultures from patients with FA with lymphocyte mosaicism, two cell populations were distinguished at 300 nM MMC, one behaving like typical FA cells, that is showing more than or equal to 10 breaks/cell, and one behaving like healthy controls, largely represented by the categories 0, 1, and 2 breaks/cell. Mosaicism was noted when at least 50% of metaphases exhibit chromosomal breakages and remaining 50% metaphases did not show any chromosomal breakages or had breaks scoring <5 (Korgaonkar *et al.*, 2010).

Statistical analysis

Microsoft excel 2010 was used for data entry, and the statistical package for social science (version 21; SPSS Inc, Chicago, Illinois, USA) was used for data analysis.

Results

A total of 25 patients with clinical suscpicion of FA were included in this study. Positive family history was noted in 7/25 (28%) of patients.

The most common presenting symptoms were bleeding tendency, in 18/25 (72%), followed by pallor in 7/25 (28%) and stunted growth in 5/25 (20%).

Clinical data of the patients are presented in Fig. 1 and Tables 1 and 2.

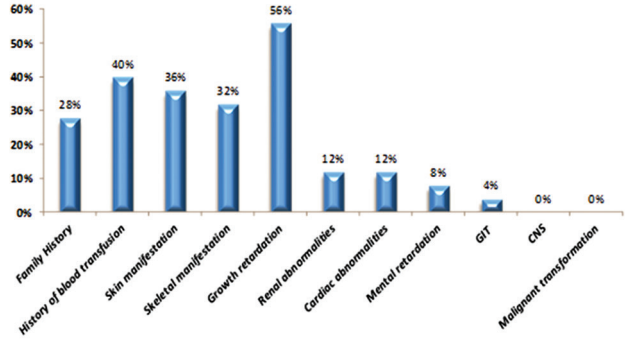
A total of 25 age-matched and sex-matched healthy volunteers, not relatives of the patients, were included in the current study as a control group.

Regarding the laboratory data, hemoglobin level ranged from 4.8 to 9.4 g/dl (mean = 6.6±1.2 g/dl), total leukocytic count ranged from 1.8 to 4.5 × 10^3/cm^3 (median = 2.5 × 10^3/cm^3), and platelet count ranged from 31 to 100 × 10^3/cm^3 (median = 65 × 10^3/cm^3). Reticulocytic count ranged from 0.1 to 0.3% (median = 0.2%). On bone marrow examination, all the patients had hypocellular bone marrow.

Descriptive data of breakage test for the patients are presented in Table 3. Chromosomal breakage test results were significantly different between patients and controls. At 0-nM MMC, all patients had negative breakage test; at 150-nM MMC, 13 (52%) out of 25 patients had a positive breakage test [5/25 (20%)], with a score ranging from 1 to 5, and eight (32%) out of 25 patients with a score more than 5; and at 300-nM MMC, all patients had a positive breakage test, where 12 (48%) of 25 patients scored 1–5, eight (32%) of 25 patients scored more than 5, whereas five (20%) of 25 patients had some metaphases, with a score of 1–5, and others with a score of more than 5. Breaks/cell ranged from 0.15 to 7 (median = 2.5). Breaks/aberrant cell ranged from 1 to 7.2 (median = 4). CFI ranged from 15 to 691 (median = 248). There was a significant relationship between chromosomal breakage test at 300-nM MMC and skin manifestation of the patients ($P < 0.01$), whereas no significant relationship with other clinical manifestations was found (Figs. 2–4).

Meanwhile, all controls showed a negative breakage test at 0-nM and at 150-nM MMC. At 300-nM MMC,

Figure 1



Clinical data of patients.

Table 1 Descriptive clinical data of the patients (N=25)

n	Age (years)	Sex	Family history and consanguinity	Presenting symptom			Duration of disease	Frequency of blood transfusion
				Pallor	Bleeding tendency	Stunted growth		
1	5	F	–	–	+	–	1 year	–
2	8	M	2 cousins with FA	–	+	–	2 years	Recurrent
3	8	F	–	–	–	+	3 years	–
4	6	M	+ve consanguinity	+	–	–	1 year	Single
5	9	M	one sister with FA	+	+	–	4 years	–
6	9	M	–	–	+	–	3 years	Twice
7	9	M	–	–	–	+	4 years	–
8	9	F	–	–	+	–	1 year	Recurrent
9	8	F	–	+	–	–	3 years	–
10	7	M	+ve consanguinity	–	+	–	1 year	Recurrent
11	7	M	–	+	+	–	1 year	Recurrent
12	18	F	–	–	–	+	12 years	–
13	8	M	–	–	+	–	2 years	–
14	6	M	–	+	+	–	2 years	Single
15	5	F	–	+	–	+	2 months	–
16	6	F	–	–	+	–	5 months	–
17	6	F	–	–	+	+	2 years	Recurrent
18	4	F	+ve consanguinity	–	+	–	4 months	–
19	7	M	–	+	–	–	2 years	Recurrent
20	10	M	–	–	+	–	4 years	–
21	8	M	–	–	+	–	1 years	–
22	5	F	One sister with FA	–	+	–	6 months	–
23	5	F	–	–	+	–	3 months	–
24	6	M	+ve consanguinity	–	+	–	1 year	–
25	10	M	–	–	+	–	3 years	Recurrent
25	10	M	–	–	+	–	3 years	Recurrent

fx1 , Fanconi group. fx2 Fanconi group. –, absent; +, present; F, female; FA, Fanconi anemia; M, male.

Table 2 Descriptive clinical data of the patients (*n*=25)

n	Skin manifestation	Skeletal abnormalities	Growth retardation	Renal abnormalities	Cardiac anomalies	Intellectual disability	Malignant transformation	Others: CNS, GIT, endocrine
1	Café au lait spots	–	–	–	–	–	–	–
2	Café au lait spots	Hypoplastic thumb	+	Absent kidney	–	–	–	–
3	Café au lait spots	Microcephaly	+	–	–	–	–	–
4	Café au lait spots	–	–	Pelvic kidney	–	–	–	–
5		–	+	–	–	–	–	–
6	Hypopigmentation	–	+	–	–	–	–	–
7	Café au lait spots	–	+	Horse-shoe kidney	–	–	–	–
8	–	Microcephaly	+	–	–	–	–	–
9	–	Microcephaly	+	–	–	–	–	–
10	–	–	+	–	–	–	–	–
11	Café au lait spots	–	–	–	VSD	–	–	–
12	–	–	+	–	–	–	–	DM
13	–	Hypoplastic thumb	–	–	–	–	–	–
14	–	–	–	–	–	–	–	–
15	–	Microcephaly	+	–	–	–	–	–
16	Café au lait spots	–	–	–	VSD	–	–	–
17	–	–	+	–	–	–	–	–
18	–	–	+	–	–	–	–	–
19	–	–	–	–	–	+	–	–
20	–	–	+	–	–	–	–	–
21	Hypopigmentation	–	–	–	ASD	–	–	–
22	–	Microcephaly	–	–	–	–	–	–
23	–	–	–	–	–	+	–	–
24	–	–	+	–	–	–	–	–
25	–	Microcephaly	–	–	–	–	–	–

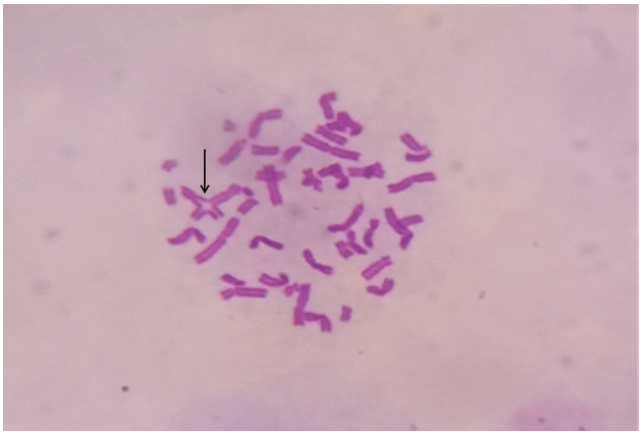
fx3 Fanconi group. fx4 Fanconi group. –, absent; +, present; ASD, atrial septal defect; VSD, ventricular septal defect.

Table 3 Descriptive data of breakage test for the patients (*n*=25)

n	Different concentrations of mitomycin C									
	150 nM				300 nM					
	% of breaks at 0 nM	% of aberrant cells at 150	Scoring		Scoring					
			1-5	>5	% of aberrant cells	Breaks/cell	Breaks/aberrant cell	CFI	1-5	>5
1	0	50	–	50	96	7	7.2	691	–	96
2	0	57	–	57	92	5.8	6.4	589	–	92
3	0	45	–	45	96	7	7.2	691	–	96
4	0	48	–	48	94	4.8	5.2	489	–	94
5	0	45	–	45	94	5.9	6.3	592	–	94
6	0	60	–	60	92	4.8	5.3	487	–	92
7	0	48	–	48	90	5.8	6.5	585	–	90
8	0	52	–	52	90	4.5	5	450	–	90
9	0	44	30	14	60	3	5	300	5	55
10	0	43	40	3	62	2.5	4	248	5	57
11	0	50	40	10	65	3.3	5	325	10	55
12	0	40	32	8	60	3.6	6	360	10	50
13	0	52	42	10	65	2.6	4	260	5	60
14	0	0	–	–	25	0.75	3	75	25	–
15	0	0	–	–	18	0.27	1.5	27	18	–
16	0	0	–	–	20	0.4	2	40	20	–
17	0	0	–	–	22	0.37	1.7	37.4	22	–
18	0	0	–	–	26	0.9	3.5	91	26	–
19	0	0	–	–	18	0.36	2	36	18	–
20	0	0	–	–	24	0.6	2.5	58	24	–
21	0	0	–	–	20	0.3	1.5	30	20	–
22	0	0	–	–	15	0.15	1	15	15	–
23	0	0	–	–	18	0.45	2.5	45	18	–
24	0	0	–	–	22	0.44	2	44	22	–
25	0	0	–	–	25	0.37	1.5	37.5	25	–

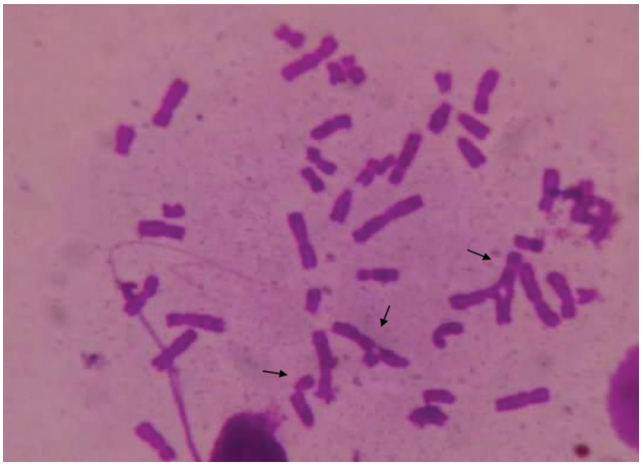
fx5 Fanconi group. fx6 Non-Fanconi group. CFI, chromosomal fragility index.

Figure 2



Case no. 11 showing quadriradial chromosomal abnormality at 150-nM mitomycin C (score 1–5).

Figure 3



Case no. 8 showing two triradial chromosomal abnormalities and a break in another chromosomes 150-nM mitomycin C (scoring >5).

all controls had breaks in less than 30% of metaphases, with all scoring 1–5. Breaks/cell ranged from 0.12 to 0.91 (median = 0.36). Breaks/aberrant cell ranged from 1 to 3.5 (median = 2). CFI ranged from 12 to 91 (median = 36).

According to the percent of breaks at 300-nM MMC, patients were divided into two groups: FA and non-FA). Comparative values of chromosomal breakage test in both groups are presented in Table 4.

According to the results of chromosomal breakage test, at 300-nM concentration of MMC, FA group was further divided into classic Fanconi anemia (CFA) (8/13), where no undamaged cells should be left, and Fanconi with mosaicism (FA-Mo) (5/13).

There was no statistical difference between patients with CFA and FA-Mo regarding their clinical data

or hemogram findings, as seen in Tables 5 and 6, but there was a significant difference between both groups regarding their chromosomal breakage test results, except for breaks at 150-nM MMC concentration ($P = 0.482$), as illustrated in Table 7.

Discussion

FA is the most frequently reported of the rare inherited BMF syndromes characterized by aplastic anemia, increased susceptibility to leukemia and cancer, and genomic instabilities. Protein products encoded by 22 FA genes, identified till date, cooperate in a molecular pathway called the FA pathway working on the repair of DNA interstrand cross-links induced by chemotherapeutic agents, such as MMC and cisplatin (Yamashita, 2019).

Chromosomal breakage test is a simple and reliable test for diagnosis of FA but needs additional studies to detect mosaic cases, which are often difficult to identify both clinically and diagnostically and may be missed (Alter *et al.*, 2005).

This gene reversion results in acquired heterozygosity in lymphocytes, rendering them less sensitive to clastogenic effects of DNA cross-linking agents. However, there is no standard degree of chromosomal breakage in peripheral blood lymphocytes that clearly differentiates patients with FA with mosaicism from

Table 4 Comparative statistical values of chromosomal breakage test in both groups

	FA group (*n*=13)			Non-FA group (*n*=12)			*P*
	Range	Median	Mean±SD	Range	Median	Mean±SD	
% of breaks at 0 nM	0	0	0	0	0	0	
% of breaks at 150 nM	43-57	45	34.9±21	0	0	0	0.000
% of breaks at 300 nM	60-96	65	60.5±38.77	15-26	20	18.1±8.3	0.000
Breaks/cell at 300 nM	2.5-7	4.8	4.67±1.58	0.15-0.9	0.39	0.45±0.21	0.000
Breaks/aberrant cell at 300 nM	4-7.2	5.3	5.6±1.1	1.0-3.5	2	2.1±0.7	0.000
CFI at 300 nM	248-691	487	466.7±157	15-91	38.8	44.66±21	0.000

CFI, chromosomal fragility index; FA, Fanconi anemia. $P<0.01$ is significant.

Table 5 Comparison between classic Fanconi anemia patients and Fanconi anemia with mosaicism regarding their clinical data

Items	Classic Fanconi anemia (*n*=8) [*n* (%)]	Fanconi anemia with mosaicism (*n*=5) [*n* (%)]	*P*
Sex:male	5/8 (62.5)	3/5 (60)	0.928 (NS)
Sex:female	3/8 (37.5)	2/5 (40)	
Skin manifestations	6/8 (75)	1/5 (20)	0.146 (NS)
Skeletal manifestations	3/8 (38)	2/5 (40)	0.928 (NS)
Growth retardation	6/8 (75)	3/5 (60)	0.569 (NS)
Renal manifestations	3/8 (38)	0/5	0.487 (NS)
Cardiac manifestations	0/8	1/5 (20)	0.188 (NS)
Mental retardation	0/8	0/5	0.125 (NS)
CNS, GIT, Endocrine	0/8	1/5 (20)	0.327 (NS)

$P>0.05$ is nonsignificant.

Table 6 Comparative statistical values of complete blood picture of classic Fanconi anemia and Fanconi with mosaicism

	Classic Fanconi anemia (*n*=8)			Fanconi anemia with mosaicism (*n*=5)			*P*
	Range	Median	Mean±SD	Range	Median	Mean±SD	
Hb (g/dl)	5-7.9	6.5	6.39±0.92	4.8-9.4	6.9	7.1±2	0.409 (NS)
TLC ($\times 10^3/cm^3$)	1.9-4.5	2.8	2.8±0.9	2.2-4.4	3.2	3.32±1	0.39 (NS)
PLT ($\times 10^3/cm^3$)	35-90	68	67.6±17.1	31-86	70	62±25.13	0.638 (NS)
Reticulocyte %	0.1-0.3	0.2	0.2±0.1	0.1-0.2	0.2	0.16±0.1	0.530 (NS)

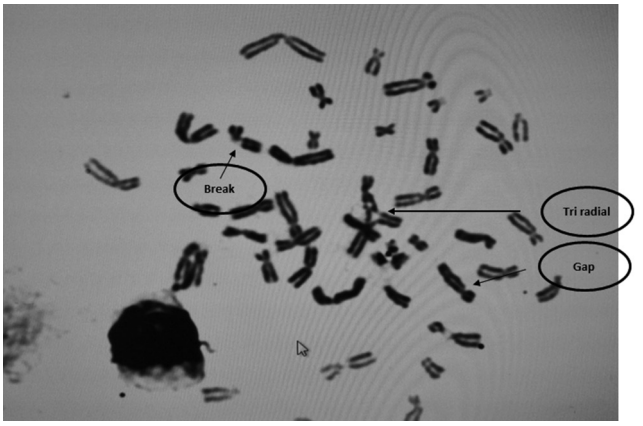
Hb, hemoglobin; TLC, total leukocytic count. $P>0.05$ is nonsignificant (NS).

Table 7 Comparative statistical values of chromosomal breakage test in classic Fanconi anemia and Fanconi with mosaicism

	Classic Fanconi anemia (*n*=8)			Fanconi anemia with mosaicism (*n*=5)			*P*
	Range	Median	Mean±SD	Range	Median	Mean±SD	
% of breaks at 0 nM	0	0	0	0	0	0	
% of breaks at 150 nM	43-57	46.5	38.3±21	0	43	29.6±23.2	0.482
% of breaks at 300 nM	60-96	93	62.7±39.4	15-26	62	40.8±31.8	0.027
Breaks/cell at 300 nM	2.5-7	5.8	5.7±0.96	0.15-0.9	3	3±0.46	0.000
Breaks/aberrant cell at 300 nM	4-7.2	6.4	6.14±0.87	1.0-3.5	5	4.8±0.84	0.000
CFI at 300 nM	248-691	587	571.8±91.2	15-91	300	298.6±46.15	0.000

CFI, chromosomal fragility index. $P>0.05$ is nonsignificant. $P<0.05$ is significant.

Figure 4



Case no. 9 showing triradial chromosomal abnormality, two gaps and two breaks at 300-nM mitomycin C (scoring > 5).

FA heterozygotes or from healthy individuals without FA (Oostra *et al.*, 2012).

Our aim was to differentiate FA mosaic cases from classical FA cases and help clarify their diagnosis in addition to correlating the results of chromosomal breakage to the patients' clinical data.

In the patient group, male: female ratio was 1.3: 1. Patients had bleeding tendency (72%), skin manifestations (36%), skeletal abnormalities (32%), growth retardation (56%), renal abnormalities (12%), and cardiac abnormalities (12%). There was a significant difference between patients and controls regarding blood counts (P = 0.001), as all patients had pancytopenia with low reticulocytic count. In an Indian study by Chowdhry *et al.* (2014), male: female ratio was 3.4: 1 in patients with aplastic anemia; the most common clinical manifestations were bleeding tendency (77.7%), skin manifestations (74.2%), and skeletal abnormalities (20.7%).

Regarding chromosomal breakage test for patients and controls, there was a significant difference between patients and controls regarding chromosomal breakage per cell (P = 0.000), chromosomal breakage per aberrant cell (P = 0.000), and the CFI (P = 0.000) at 300-nM MMC concentration. These results were in concordance with the study done by Temtamy *et al.* (2007), which was also conducted on Egyptian patients using the DEB technique, where 70% of the FA cases had cells that showed more than 10 breaks/cell, whereas the non-FA group showed no such aberrations in any of their cells. Our results are also in agreement with results from the study by Oostra *et al.* (2012). In contrast, Korgaonkar *et al.* (2010), recorded an elevated percentage of spontaneous chromosomal breakage in up to 63% of patients at 0-Nm MMC. However, Wu (2016), stated that spontaneous chromosomal breakage is less common, but it correlates with patient clinical severity.

In the current study, a significant relation between chromosomal breakage test at 300-nM MMC and skin manifestations of the patients (P = 0.066) was suggested; however, correlation with other clinical data was insignificant. Ricardo *et al.* (2011), in also recorded an insignificant relation between chromosomal breakage test and clinical data of their patients.

Patients were divided into FA and non-FA groups according to the results of chromosomal breakage test at 300-nM MMC concentration. In the FA group, 69% had growth retardation, 54% had skin manifestations, 38% had skeletal manifestations, renal abnormalities in 24% of patients, and cardiac abnormalities in 8% of patients, which were in agreement with Korgaonkar *et al.* (2010).

For the chromosomal breakage test in FA group, patients had a positive breakage test result at 150- and 300-nM MMC concentration, which significantly differed from non-FA group. At 300-nM MMC, the mean chromosomal breakage per cell was 4.67 ± 1.58 (P = 0.000), the mean chromosomal breakage per aberrant cell was 5.6 ± 1.1 (P = 0.000), and the mean CFI was 466.7 ± 157 (P = 0.000), which significantly differed from non-FA group. Temtamy *et al.* (2007), detected a wide range of breaks/cell in Egyptian FA cases, ranging from 1.2 to 12.1, with a mean of 4.3 breaks/cell. In response to adding MMC, all patients with FA exhibited the characteristic breaks in many of their cells. Overall, 90% of the parents revealed an increase of chromosomal breakage using DEB but not MMC. Although the results of the parents were statistically significant, this led to an overlap between the parents and the controls. The spontaneous chromosomal break results revealed that not all the bands are randomly involved as most of the bands were correlated with either fragile sites or oncogenes and/or cancer break points. Fargo *et al.* (2014), compared chromosomal breakage test in patients with CFA, patients with FA with mosaicism, and patients with other inherited BMF syndromes, where chromosomal breaks per cell were found to be 6, chromosomal breaks per aberrant cell were 7, and the CFI was 593.

At 300-nM MMC, patients with FA were further divided into CFA and FA-Mo, where 38% of patients with FA showed lymphocyte mosaicism, which was considered a high percentage compared with other studies, where 25% of FA cases showed mosaicism (Soulier *et al.*, 2005).

Previous research studies reported that mosaics were lower than CFA in number of breaks per cell. The number of breaks per aberrant cell was statistically lower in mosaics compared with CFA, with an obvious overlap. The CFI was lower in the mosaics than patients with CFA. They stated that patients with mosaicism might be distinguished from CFA based on percentage of aberrant cells, breaks per cell, and CFI, but not breaks per aberrant cell, in which there was a small amount of overlap (Fargo *et al.*, 2014).

Moreover, the study by Korgaonkar *et al.* (2010), reported that mosaic cases had much higher values for mean chromosomal breakage per cell, chromosomal breakage per aberrant cell, and CFI compared with controls and lower values compared with the CFA cases without any overlap.

There were no significant differences between both groups regarding any clinical or laboratory data except for results of chromosomal breakage test, which was also noted by Fargo *et al.* (2014).

It is recommended that this study is applied on a larger number of patients in each group for more guided results. Molecular studies are also recommended for confirmatory diagnosis of suspected FA cases and determination of the affected genes.

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Conflicts of interest
There are no conflicts of interest.

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