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*Original scientific paper*

# COMET ASSAY AND CYTOGENETIC FINDINGS IN DIFFERENTIAL DIAGNOSIS OF FANCONI ANEMIA

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Fanconi anemia (FA) is a complex genetic disease with a variety of congenital and hematological symptoms, including the predisposition for cancer development. The main hallmark of FA cells, an increased chromosomal fragility, in the presence of the DNA-interstrand cross-linking chemicals, mitomycin C or diepoxybutane (DEB), makes the

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diagnosis of FA much easier. Cytogenetic method can detect the FA patients with highly elevated chromosomal breakage, but also some of the patients with borderline sensitivity to DEB no matter if they have FA or not. These particular circumstances lead us to introduce comet assay along with cytogenetic analysis, in order to determine DNA lesions and chromosomal fragility in untreated and DEB-treated lymphocytes of full blood from seven patients with clinical features of FA. Highly elevated DEB induced chromosomal sensitivity confirmed the diagnosis in five patients (FA group: 0.48-4.47 breaks/cell vs control group: 0.00-0.08 breaks/cell). Borderline DEB sensitivity (FA* group: 0.15-0.44 breaks/cell) was found in the remaining two patients. Results of the comet assay showed higher baseline and DEB-induced DNA damage values (Olive tail moment and tail intensity) in all five FA and one FA* patient, when compared to the control group. These findings could provide a new model of FA screening test algorithm, including comet assay as additional and very useful accurate tool, beside the DEB test, in differential diagnosis of FA.

*Keywords*: Fanconi anemia, genetic disease, comet assay, chromosomal fragility, diepoxybutane

## INTRODUCTION

Fanconi anemia (MIM ID #227650) (FA) is a rare polygenic chromosome breakage disorder, with progressive pancytopenia and bone marrow failure, variable congenital anomalies and strong susceptibility for malignancy (TISCHKOWITZ and HODGSON, 2003; BAGBY *et al*., 2004; ALTER and KUPFER, 2014). Diagnosis of FA on the basis of clinical features is difficult due to the possible overlap of FA phenotype with the variety of genetic and non-genetic bone marrow failure (BMF) syndromes (ALTER, 2008). FA is both clinically and genetically very heterogeneous disease, as 16 different FA complementation groups (genes) have been identified so far (A, B, C, D1 (*BRCA2*), D2, E, F, G, I, J (*BRIP1*), L, M, N (*PALB2*), O (*RAD51C*), P (*SLX4*) and Q (*ERCC4*)) (WANG, 2007; ALTER, 2008; MOLDOVAN and D'ANDREA, 2009; VAZ *et al*., 2010; STOEPKER *et al*., 2011; ALTER and KUPFER, 2014; KOHLHASE *et al*., 2014). It is well known that these genes are involved in DNA repair mechanisms, so its mutations lead to genome instability and clinical symptoms of the disease (HISAMA *et al*., 2003). The common characteristic of FA cells is an increased level of spontaneous chromosomal fragility, which could be induced with mutagens such as mitomycin C (MMC), diepoxybutane (DEB) and other agents, thanks to their alkylating and DNA cross-linking ability (AUERBACH, 2004; OOSTRA *et al*., 2012). Hypersensitivity of FA cells to interstrand crosslinks (ICL) inducing agents (DEB and MMC tests) is widely used for screening of FA patients among patients with BMF syndromes (AUERBACH, 2004).

The comet assay is widely regarded as a fast and informative method for the measurement of primary DNA damage at the single-cell level (SINGH *et al*., 1988). This technique, first described by OSTLING and JOHANSON (1984) and in a modified alkaline version by SINGH *et al*. (1988), allows easy and fast visualization and measurement of DNA damage on the whole genome level of single cells. It should be noted that DNA lesions consisting of strand breaks, after treatment with alkali, either alone or in combination with certain enzymes (e.g. endonucleases) increase DNA migration compared to those in concurrent controls (TICE *et al*., 2000). Research on DNA damage induced by agents such as ionizing radiation, environmental toxins, DNA binding substances or therapeutics has been performed during the last years with

the comet assay (MOHSENI-MEYBODI and MOZDARANI, 2009; AZQUETA *et al*., 2014; GLEI *et* al., 2016). Unlike classical chromosome breakage, micronucleus formation or cell survival studies, the comet assay measures DNA damage strand breaks and alkali-labile sites after exposure with various agents (DJUZENOVA *et al*., 2001). The comparison of the intensities of tail DNA and head DNA under a conventional fluorescence microscope allows quantification of DNA damage, which can be performed with automated software systems (GYORI *et al.*, 2014).

In the present study we investigate spontaneous and DEB induced sensitivity of FA cells measured at the chromosome level (DEB test) and the single cell level (comet assay).

## MATERIAL AND METHODS

*Participants*

Participants included in this study were seven patients with aplastic anemia (AA), BMF and other symptoms of FA, as well as healthy individuals, who visited the Mother and Child Health Care Institute of Serbia "Dr Vukan Cupic" (MCHCIS) and the University Children's Hospital from September 2009 to January 2012. They all underwent DEB test on peripheral blood samples in the Laboratory for medical genetics at the MCHCIS which was approved by the Ethics Committee of MCHCIS. Amongpatients, six were children (≤18 years with mean age at diagnosis 13.1 years) and one was an adult (36 years). In this group of patients, females were more frequent (5) than males (2).

*Methods*

*Cytogenetic analysis*

*DEB test*

Cytogenetic analysis of chromosome fragility was performed on peripheral blood lymphocytes from patients suspected of FA and from controls (healthy persons) as previously described, with some modification (AUERBACH, 2004). Six cultures were obtained from each of the seven patients as well from the seven healthy individuals (controls) too, and DEB (CAS No. 1464-53-5) was added to four of them in the final concentration of 0.1μg/mL for the last 24h and 48h (kept in the dark), while the remaining two cultures were left untreated (AUERBACH, 2004). Colcemid (2.5 μg/mL) was added to all cultures two hours before the preparation, which was performed according to standard procedure (AUERBACH, 2004). Chromosome fragility analysis was conducted on 50-100 Gimsa stained metaphases from each culture and each patient and control was karyotyped by G banding technique (AUERBACH, 2004; ISCN, 2009). The simultaneous presence of chromosome/chromatid break and acentric fragment was counted as one break, while other aberrations such as ring and dicentric chromosome were counted as two breaks (AUERBACH, 2004). Radial structures were counted according to the number of chromosomes/centromeres involved in it (two and more breaks) (AUERBACH, 2004; OOSTRA *et al*., 2012). The chromosomal instability was evaluated and presented as percentage of aberrant cells and the number of breaks per cell (AUERBACH, 2004).

*Alkaline comet assay*

The alkaline comet assay procedure was performed under yellow light, basically using the method described by SINGH *et al*. (1988). Microscopic slides were precoated with 1% NMP (normal melting point agarose, Sigma-Life Science) and air dried for 24h. To form the second, supportive layer, 80μL of 1% NMP agarose was gently placed on the top of the first NMP layer

and spread over the slide using coverslip. The slide was placed on ice for 5 min to allow to complete polymerization of the agarose. After the coverslips were removed, 30μL of lymphocytes pellet suspension obtained from the full peripheral blood, gently mixed with 70μL of 1% LMP (low melting point agarose, Sigma-Life Science), was pipetted on the supportive layer of 1% NMP agarose and covered with a coverslip. After keeping coverslips for 5 min on ice, they were removed and the slides were placed into freshly made cold lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1.5% Triton X-100, pH 10) for 1 hour. To allow DNA unwinding, slides were put into the electrophoresis chamber containing cold alkaline electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH 13) for 20 min. Electrophoresis was performed at 0.75 V/cm at 4ºC for 20 min. After the electrophoresis, the slides were placed into freshly made neutralizing buffer (0.4 M Tris, pH 7.5) for 15 min. Staining was performed with 20 μL per slide of EtBr (2 μg/mL). The slides were examined with a fluorescence microscope (Leica, DMLS, Austria, 400 × magnifications, 510-560 nm excitation filter, and 590 nm barrier filter). Microscopic images of comets were scored using the Comet IV Computer Software (Perceptive Instruments, UK). Images of 50 cells were captured from each slide per sample and among the parameters available for analyses, the tail intensity (TI) and Olive tail moment (OTM) were chosen, as parameters to assess the DNA damage,Values were expressed as mean value ± SD.

*Statistical analysis*

We used the Chi-square test to determine a difference between the groups of aberrant cells from each patient and its control counterpart (AUERBACH, 2004). According to the sensitivity to DEB (values of percentage of aberrant cells and breaks per cell) patients were divided into two subgroups: FA and borderline FA*.

One-way analysis of variance (ANOVA) was applied to compare the differences of mean tail intensity and Olive tail moments among different samples. p value of less than 0.05 was considered as significant level.

## RESULTS

*Chromosome breakage analysis*

Seven patients with clinical suspicion of FA were evaluated for chromosome breakage, by using the DEB test. The chromosome breakage was determined according to two main parameters: “percentage of aberrant cells” and “breaks/cell”. DEB test revealed two subgroups of patients: FA – with DEB sensitive cellular response and FA* - with borderline sensitivity to DEB. The results for these two groups (FA and FA*) obtained on spontaneous and DEB-induced breakage are summarized in Table 1. In the group of seven examined patients five (71.4%) of them were found to have FA characteristic increased number of DEB-induced chromatid/chromosome breaks, acentric fragments, ring chromosomes and other chromatid/chromosome inter and intrachanges (Chi square test: $p < 0.05$) (Figure 1, Table 1). Two (28.6%) out of seven patients showed borderline values of percentage of aberrant cells (Chi square test: $p > 0.05$) and number of breaks/cell.

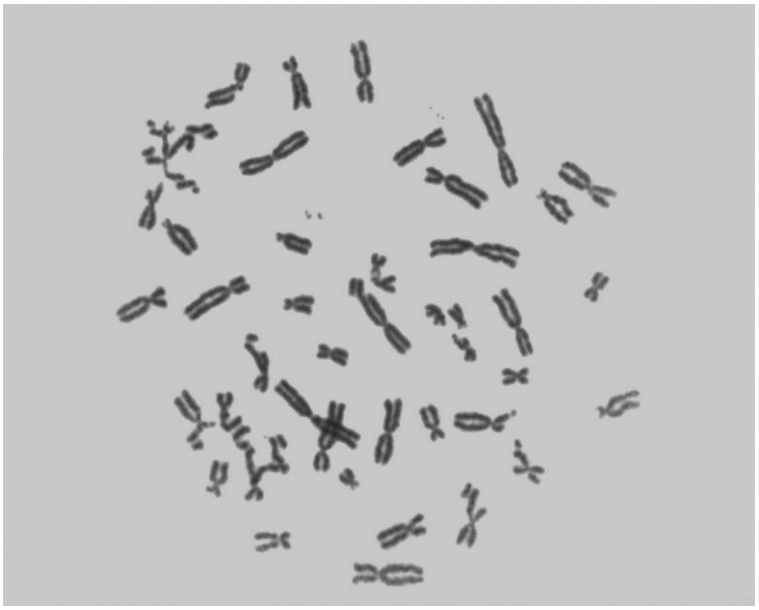

Figure 1. Multiple DEB-induced chromosome aberrations in FA patient

*Table 1. Evaluation of spontaneous and DEB-induced chromosome breakage findings in Fanconi anemia (FA), borderline-FA (FA*) and control groups.*

| **Group** | **N** | **Range** | |
|---|---|---|---|
| | | **N° breaks/cell** | **Aberrant cells (%)** |
| ***Spontaneous chromosome fragility*** | | | |
| FA | 5 | 0,03 – 0,39 | 3 – 29 |
| FA* | 2 | 0,06 – 0,12 | 5 – 12 |
| Controls | 7 | 0,00 – 0,03 | 0 – 3 |
| ***DEB induced (24h) chromosome fragility*** | | | |
| FA | 5 | 0,48 – 4,39 | 35 – 82 |
| FA* | 2 | 0,15 – 0,26 | 13 – 22 |
| Controls | 7 | 0,00 – 0,08 | 0 – 8 |
| ***DEB induced (48h) chromosome fragility*** | | | |
| FA | 4 | 0,56 – 4,47 | 38 – 86 |
| FA* | 2 | 0,20 – 0,44 | 14 – 28 |
| Controls | 6 | 0,02 – 0,06 | 2 – 4 |

The percentage of 24h-DEB-induced aberrant cells in five FA patients ranged from 35% to 82%, which was about ten times higher than it was in the control group (range: 0% - 8%) (Table 1). According to the percentage of aberrant cells induced with DEB for 48h in the FA group, there was a trend of increased values, in comparison to the 24h-treatment, with some

exceptions (percentage of aberrant cells of patient No. FA-105: 64% (24h) vs 38% (48h)) (Table 2). When the number of breaks/cell was analyzed, the interval of values for this parameter in the FA group was much higher when compared to the control group (FA group: 0.48 to 4.39 breaks/cell vs control group: 0.00 - 0.08 breaks/cell) (Tables 1 and 2).

*Table 2. Chromosome fragility of five FA patients*

| **Nº patient** | **Gender** | **Age at diagnosis** | **Spontaneous chromosome fragility** | | **DEB-induced (24h) chromosome fragility** | | **DEB-induced (48h) chromosome fragility** | |
|---|---|---|---|---|---|---|---|---|
| | | | *Nº breaks/ cell* | *Aberrant cells (%)* | *Nº breaks/ cell* | *Aberrant cells (%)* | *Nº breaks/ cell* | *Aberrant cells (%)* |
| *FA*-13 | female | 18 | 0.08 | 8 | 0.68 | 35 | / | / |
| *FA*-65 | female | 16 | 0.39 | 29 | 4.39 | 82 | 4.47 | 86 |
| *FA*-83 | male | 12 | 0.03 | 3 | 0.91 | 37 | 1.40 | 50 |
| *FA*-98 | female | 14 | 0.12 | 9 | 0.48 | 40 | 1.60 | 48 |
| *FA*-105 | female | 10 | 0.22 | 17 | 1.31 | 64 | 0.56 | 38 |
| | *Range* | | **0.03 - 0.39** | **3 - 29** | **0.48 - 4.39** | **35 - 82** | **0.56 - 4.47** | **38 - 86** |

*Table 3. Chromosome fragility of two FA*-borderline patients*

| **Nº patient** | **Gender** | **Age at diagnosis** | **Spontaneous chromosome fragility** | | **DEB-induced (24h) chromosome fragility** | | **DEB-induced (48h) chromosome fragility** | |
|---|---|---|---|---|---|---|---|---|
| | | | *Nº breaks/ cell* | *Aberrant cells (%)* | *Nº breaks/ cell* | *Aberrant cells (%)* | *Nº breaks/ cell* | *Aberrant cells (%)* |
| *FA**-102 | female | 9 | 0.06 | 5 | 0.26 | 22 | 0.20 | 14 |
| *FA**-110 | male | 36 | 0.12 | 12 | 0.15 | 13 | 0.44 | 28 |
| | *Range* | | **0.06-0.12** | **5 - 12** | **0.15-0.26** | **13 - 22** | **0.20-0.44** | **14 - 28** |

Age at diagnosis had no influence to the degree of DEB-24h induced fragility of FA and FA* cells. Conversely, the younger patients (FA*-102, FA-105, FA-83) showed lower values of 48h DEB-induced break/cell comparing to some older patients (FA-65, FA-98) (Tables 2 and 3).

The spontaneous chromosome fragility (percentage of aberrant cells and breaks/cell) values in five FA patients (range 3% - 29% of aberrant cells) were overlapping those in the control group (range 0 - 3% of aberrant cells) (Table 1). Nevertheless, two patients who were classified into the FA*-borderline group showed a minimum increase of number of DEB-induced aberrant cells and breaks/cell, in comparison to spontaneous chromosome breakage in these patients (spontaneous chromosome breakage: 5% - 12% of aberrant cells vs 24h-DEB-induced: 13% - 22% and 48h-DEB-induced chromosome breakage: 14% - 28%) (Table 3). There was slightly increased chromosome fragility after 48h of exposure to DEB in these patients, but not statistically significant when compared to the control group (Table 1).

*Alkaline comet assay*

In order to test cytogenetic findings, we carried out the alkaline comet test on untreated and DEB-treated (24 and 48 hours) peripheral blood lymphocytes cultures from FA, borderline FA* patients and healthy individuals (control group). Control group was divided into two subgroups, according to the age of the participants; one consisted of children (aged 9-18 years) and one of an adult (aged 36 years) (Table 4). Five FA patients (FA- 83, 98, 13, 105, 65) aged from 10 to 18 years were compared with the children control group, while two borderline patients, FA* 102 (9 years old) and FA* 110 (36 years old), were compared with the children and adult control groups, respectively.

*Table 4. Response of two control groups to treatment (0h, 24h and 48h) with DEB expressed in OTM and TI (min-max)*

| controls | OTM | | | TI | | |
|---|---|---|---|---|---|---|
| | 0h | 24h | 48h | 0h | 24h | 48h |
| children | 0.94 - 1.85 | 1.40 - 2.28 | 1.35 - 2.92 | 4.80 - 8.42 | 7.00 - 11.03 | 8.33 - 15.27 |
| adults | 1.70 - 1.85 | 0.85 - 1.82 | 2.04 - 2.50 | 9.05 - 10.36 | 5.99 - 10.90 | 8.98 - 12.25 |

Figure 2. presents basal and DEB-induced DNA damage of five FA patients and children control group, expressed with mean OTM and TI. All cytogenetically confirmed FA patients revealed substantial increase of OTM and TI at the comet assay. This was significantly different in comparison to the control group, either for spontaneous and/or DEB-induced (24h and 48h) DNA damage. When comparing results for OTM, all FA patients (except FA-83) showed higher baseline values than the control. After treatment with DEB, there was a trend toward an increased DNA damage level in the cells from FA patients. Four FA patients (65, 83, 98 and 105) showed significant increase of DNA damage level compared to the control for 24h DEB treatment, while four FA patients (13, 65, 83 and 98) showed significant increase compared to the control for 48h DEB treatment. Results of TI were slightly different. Namely, all FA patients without treatment and after 24h and 48h of treatment with DEB showed significant increase in DNA damage, except for FA 105, where increase was notable only in untreated cells.

Comet assay method was also sensitive regarding FA* borderline patients for both OTM and TI (Figure 3). Results of OTM showed that one (FA* 110) of two borderline patients had values in the range of FA patients. FA* 110 was significantly different compared to adult control group for 0h and 24h ($p<0.05$), while FA* 102, did not show difference in comparison to the children control group, neither for spontaneous nor DEB-induced (24h and 48h) DNA damage. Analysis of results with TI of two borderline patients showed significant increase of DNA damage, both at 0h and after incubation with DEB for 24h ($p<0.05$).

In this study was observed that that from the group of FA and FA* patients (two males and five females), males (FA* 110 and FA 83) had a higher response to the DEB test than females. Female group showed a gradient of damage, which was the highest in the oldest patients (FA-65 and FA-13, 16 and 18 years old, respectively) and lowest in the youngest patients (FA-105 and 102, 10 and 9 years old, respectively) according to the 48h induction time with DEB.

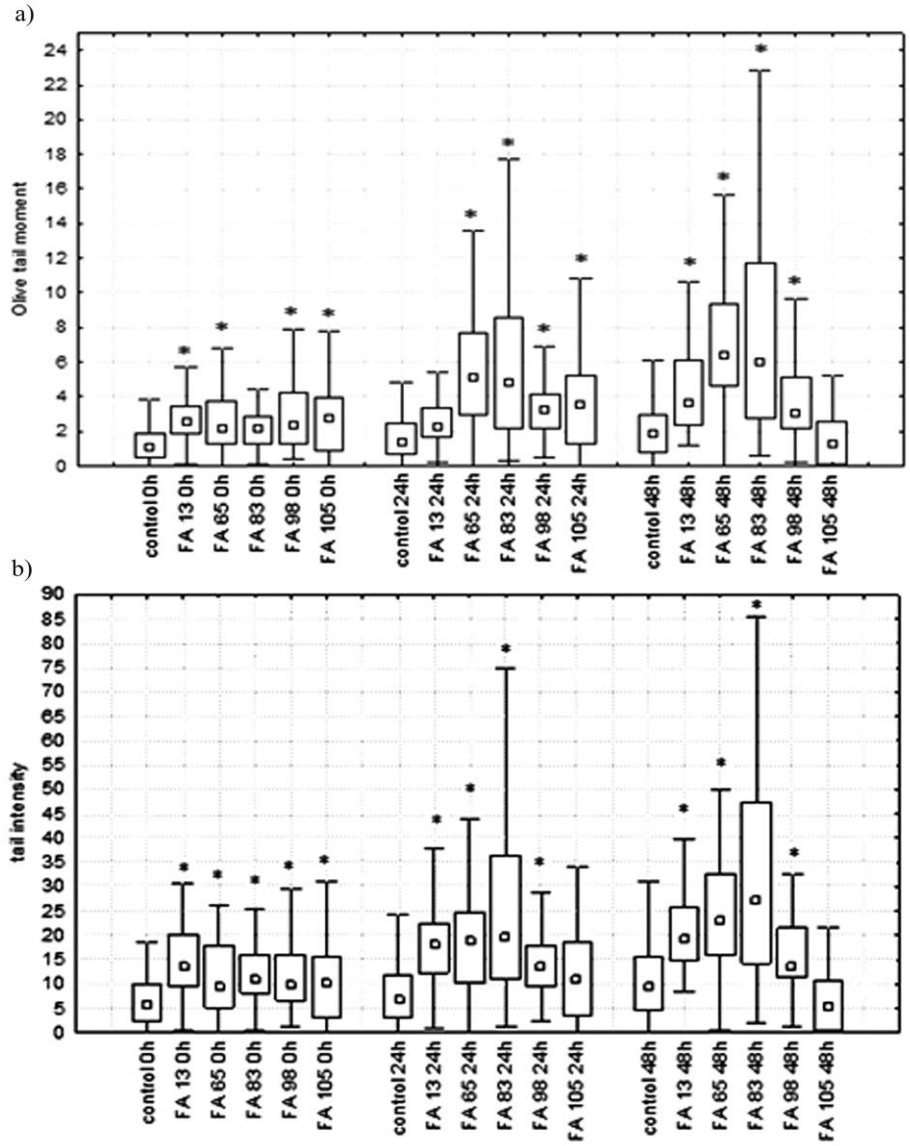


Figure 2. Response of five FA patients and control group to treatment (0h, 24h and 48h) with DEB. a) Olive tail moment b) tail intensity *Significantly different from control ($p<0.05$).

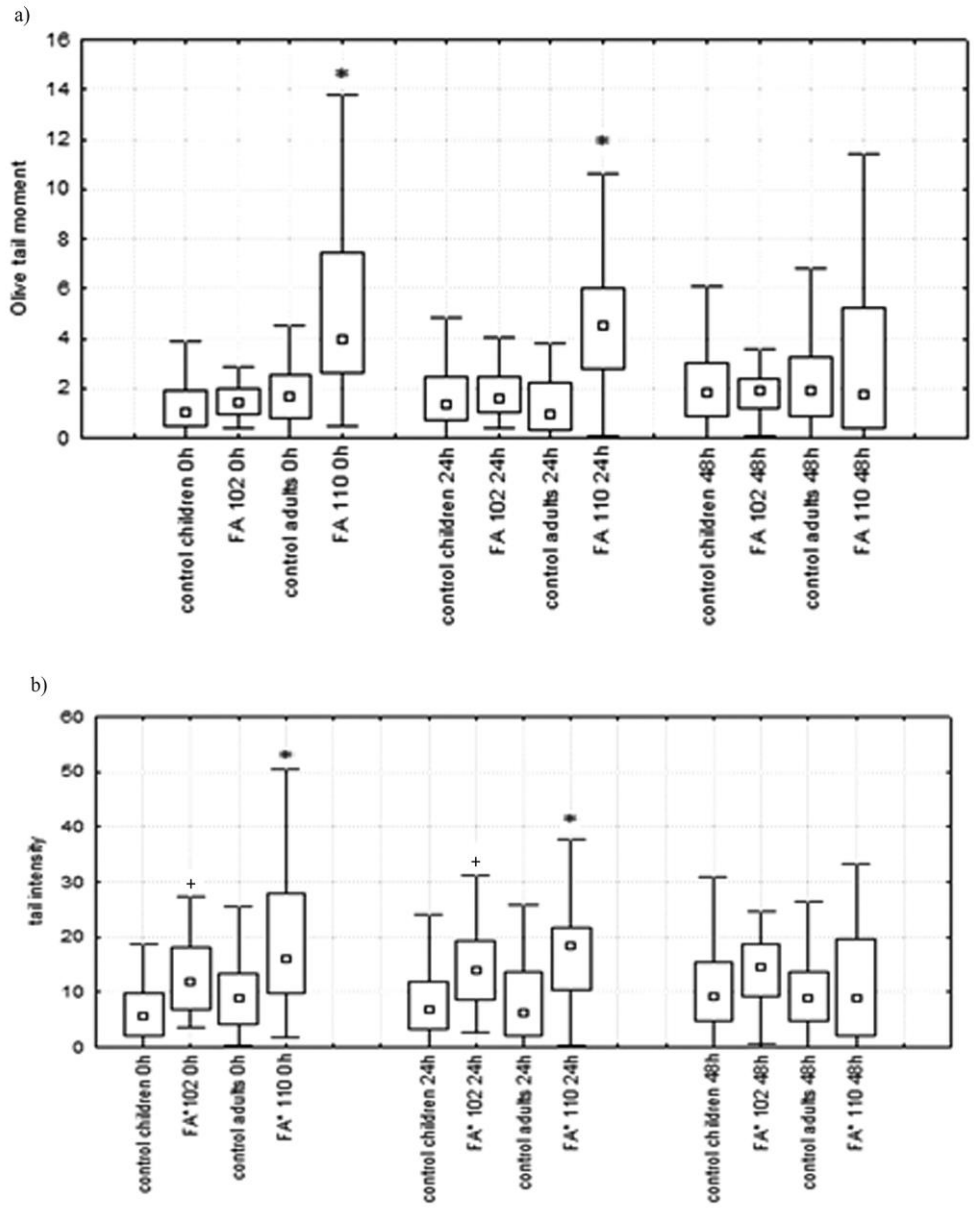


Figure 3. Response of two borderline *FA patients and two control groups to treatment (0h, 24h and 48h) with DEB a) Olive tail moment b) tail intensity $^{+}$*Significantly different from corresponding control ($p<0.05$)

## DISCUSSION

Diagnosis of FA is often very difficult to achieve due to the heterogeneity of its clinical symptoms and genetic background (TISCHKOWITZ and HODGSON, 2003; MALRIC *et al*., 2015). This is the reason why analysis of chromosome fragility (induced by DEB or MMC) is a widely used test for the diagnosis of such a heterogenic disease (ALTER, 2008; ADA *et al*., 2011; OOSTRA *et al*., 2012; YOON *et al*., 2014). The comet assay is among the most sensitive methods available for measuring primary DNA damage; it has advantages of speed, simplicity and specificity. It is also an invaluable tool for investigating fundamental aspects of DNA damage and cellular responses to this damage (COLLINS, 2004). Here we report the results of the DEB-induced chromosome fragility test in patients with suspicion of FA from the two of the largest children hospitals in Serbia.

According to chromosome fragility findings induced by DEB, the percentage of aberrant cells of FA group varied from 32% to 86% (24h and 48h of inductions in total), with no overlap compared to the same ranges of healthy individuals (0% - 8%). These results are in consistence with the results of the International Fanconi's Anemia Registry (IFAR) study (12.6% - 100%) (AUERBACH *et al*.,1989) and other similar data (KOOK *et al*., 1998; ILGIN *et al*., 1999; AUERBACH, 2004). The values of DEB-induced break/cell of FA patients (0.48 - 4.47 breaks/cell for both 24h and 48h of induction in total) were also much higher than the same values from the control group (0.00 - 0.08 breaks/cells). Our results showed the lower range of DEB-induced breaks/cell in FA patients compared to those published in IFAR study (1.30 – 23.90 breaks/cell) (AUERBACH *et al*., 1989), but they were in accordance with results of other similar studies (CASTELLA *et al*., 2011; YOON *et al*., 2014). The reason for that could be the presence of mosaic FA patients in the examined group of patients (LO TEN FOE *et al*., 1997; SOULIER *et al*., 2005; CASTELLA *et al*., 2011). In previous published data, FA patients with 0.31 – 1.52 breaks/cell and 10% - 40% of aberrant cells were considered to be mosaic, while non mosaic FA patients showed the number of break/cell in range from 1.38 to 10.00 with >60% of aberrant cells (CASTELLA *et al*., 2011). In our study, most of the FA patients could be classified as mosaic, except those whose percentage of aberrant cells was exceeding 60%. The existence of the borderline DEB-induced fragility in two of examined patients could be explained as a somatic mosaicism phenomenon, too. These patients, so called borderline FA* group, showed percentage of DEB-induced aberrant cells (13% - 28% for both 24h and 48h inductions in total) which was in the range of FA mosaic patients according to CASTELLA *et al*. (2011). But, the number of break/cell (0.15 – 0.44 for both 24h and 48h inductions) in our study was lower than those characteristic for mosaic FA patients given in CASTELLA *et al*. (2011). All these patients suspected to be FA mosaics should be referred to further investigation for final confirmation of diagnosis of FA (SOULIER *et al*., 2005; ADA *et al*., 2011).

The spontaneous chromosomal fragility (percentage of aberrant cells and number of breaks/cell) values, in our study, in FA and FA* groups of patients, were overlapping. According to the findings of the IFAR group, where spontaneous chromosomal breakage of FA (0.02 - 1.90 breaks/cell) and non FA patients (0.00 - 0.12 breaks/cell) was overlapping, the baseline breakage is expected to be various and unreliable in differential diagnosis of FA (AUERBACH, 2004). The results of our study were consistent with similar previous findings (KOOK *et al*., 1998; ILGIN *et al*., 1999; AUERBACH, 2004; YOON *et al*., 2014).

Because of the management of affected patients and development of the disease, it is very important to establish and confirm diagnosis of FA very early and with some certainty

(YOON *et al*., 2014). For that reason, authors introduced additional DNA damage testing as an effort to make diagnosis of FA more accurate and easier.

Comet assay revealed substantial increase of mean OTM and TI for untreated FA samples compared to controls as well as for DEB-treated. It could be argued that pre-existing DNA damage, as indicated by increased spontaneous chromosome breakage rates in FA, may be responsible for the high initial DNA damage in patients' untreated cells (MOHSENI-MEYBODI *et al*., 2009). Not only that comet assay confirmed the FA phenotype, but it also singled out FA* borderline patients. OTM-singled out one borderline patient (FA 110), while the TI singled out both borderline patients (FA 110 and FA 102), in comparison to the control and in the range of OTM and TI obtained in FA patients.

After treatment with DEB, FA patients generally showed an increase in DNA damage, which was in accordance with previous studies investigating FA patients treated with various agents (DJUZENOVA *et al*., 2004; MOHSENI-MEYBODI and MOZDARANI, 2009; MOHSENI-MEYBODI *et al*., 2009). Reported papers on Fanconi radiosensitivity analyzed with comet assay were often in conflict (MOHSENI-MEYBODI *et al*., 2009), when compared with similar studies using chemical agents like MMC or DEB (MOHSENI-MEYBODI and MOZDARANI, 2009).

## CONCLUSION

In this study we have shown that comet assay, beside the chromosome breakage test, could be a very useful additional tool in establishing diagnosis of FA. It represents a possible solution for investigation of the level of DNA damage, in order to confirm the FA phenotype, especially when cytogenetic testing shows borderline or no response to DEB in patients with FA. Chromosome breakage analysis in combination with comet assay could provide a more accurate, efficient and rapid confirmation of FA, which is crucial for further treatment of the patients before referring them for molecular analysis.

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# KOMET TEST I CITOGENETSKI METOD U DIFERENCIJALNOJ DIJAGNOZI FANKONI ANEMIJE

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Izvod

Fanconi anemija (FA) je kompleksna genetska bolest sa raznim kongenitalnim i hematološkim simptomima, uključujući predispoziciju za razvoj raka. Glavna karakteristika FA ćelija je povećana hromozomska nestabilnost u prisustvu DNK-interkalirajućih agenasa, kao što su mitomicin C ili diepoksibutan (DEB), usled čega je dijagnoza FA mnogo lakša. Citogenetska metoda može da izdvoji pacijente sa povišenom hromozomskom nestabilnošću, ali i pacijente sa graničnom osetljivošću na DEB. Ove posebne okolnosti dovode nas do uvođenja komet testa zajedno sa citogenetskom analizom kako bi se utvrdile DNK lezije i hromozomska nestabilnost u netretiranim i DEB tretiranim limfocitima kod sedam pacijenata sa kliničkim karakteristikama FA. Visoko povišena DEB indukovana hromozomska osetljivost potvrdila je dijagnozu u pet grupa u poređenju sa kontrolnom grupom (FA grupa: 0.48-4.47 preloma / ćeliji nasuprot kontrolama: 0.00-0.08 preloma / ćeliji). U preostala dva pacijenta nađena je granična DEB osetljivost (FA* grupa: 0,15-0,44 preloma / ćeliji). Rezultati komet testa pokazali su više bazne i DEB-indukovane vrednosti oštećenja DNK (moment repa komete i intenzitet repa) u svih pet FA i jednog FA* pacijenta, u poređenju sa kontrolnom grupom. Ovi rezultati mogli bi da obezbede novi model algoritma FA testiranja, uključujući i komet test kao dodatni, veoma koristan i precizan metod, pored DEB testa, u diferencijalnoj dijagnozi FA.

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