Letter to the Editor

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Comment on the Paper by Safi Oz et al. Entitled 'Evaluation of Micronuclei, Nuclear Anomalies and the Nuclear/Cytoplasmic Ratio of Exfoliated Cervical Epithelial Cells in Genital Candidiasis'

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Dear Editor,

I read with interest the paper of Safi Oz et al. [1]. Since our group also studied micronuclei (MN) in exfoliated human epithelial cells we have taken a special interest in the findings.

The main message of the study is that genital candidiasis is a genotoxic factor which can increase the rate of cells with MN 4.4-fold. The authors wrote in the Introduction that some infectious agents increase the number of MN in epithelial cells. While this is true for viruses and some parasites, e.g. *Schistosoma*, the genotoxic activity of *Candida albicans* is not known. Recently Reis Campos et al. [2] also reported about the increased rate of MN in cervix cells of Brazilian women with candidiasis (number of MN not specified).

In our opinion, MN induction in exfoliated cervical cells occurs not because of *Candida* infection but due to the use of inappropriate staining of exfoliated epithelial cells. Safi Oz et al. [1] used Papanicolaou staining for cervical cells, and Reis Campos et al. [2] used Giemsa staining.

The staining method has a strong impact on the outcome of MN studies with exfoliated epithelial cells [3–5]. It has been shown with buccal cells that the MN rates are overestimated with the use of non-DNA-specific stains, and it has been postulated that these misleading results are a consequence of staining of keratin bodies which have the same morphological characteristics as MN when non-DNA-specific stains are used [3, 4]. It is known that the cervix epithelium contains different types of keratins (namely 1, 6, 15, 16, and 20) [6]. Therefore, the use of stains that are non-DNA specific may lead also in this cell type to an overestimation of the MN rates. It is, furthermore, well known that keratin is produced in all types of epithelial cells that are under stress which can be induced by infection and mechanical or chemical stimuli [7]. One such stimulus can be *Candida* infection inducing of oxidative stress in cervical cells.

In the framework of the ANSEF Award (USA) we investigated in 2000–2003 MN in cervical cells of gynecological patients. In our study [8], we found an increased rate of MN in patients with cancer of the cervix uteri and in HPV-infected women who did not have cancer. In addition to the two aforementioned groups, we also analyzed exfoliated cells from 25 women with genital candidiasis [in preparation]. Four slides were prepared from cells of each woman and cells were stained with Giemsa (8%) and Feulgen/fast green (2 slides each). As a minimum, 2,000 cells were evaluated from each subject. In Giemsa-stained slides, we found a 2-fold increase in the number of cells with MN in patients with infection in comparison to the control group (3.20 ± 1.42 vs. $1.60 \pm 0.64‰$, $p < 0.02$). In cells stained with Feulgen/fast green, no significant difference was found between infected patients and controls (1.20 ± 0.64 vs. $1.00 \pm 0.46‰$, $p > 0.05$).

Hence, we think that the significant difference in rates of MN cells in infected women compared to the controls is due to the use of inappropriate stain. In the protocols concerning investigation of MN in buccal cells published recently [9, 10], it is clearly indicated that MN should be studied in 2,000 cells stained with DNA-specific stain (preferably Feulgen/fast green which gives the possibility to monitor MN both under bright light and fluorescent microscopes). It is also suggested to evaluate about 4,000 epithelial cells to obtain reliable results [11]. In the study by Safi Oz et al. [1] only 1,000 cells were evaluated, and this is not enough for this type of cells (exfoliated epithelial). It is also noteworthy that the morphology of all nuclear anoma-

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lies is the same in all types of epithelial cells [8, 12, 13].

Another unusual aspect of the study is the number of cells with MN which is extremely low compared with the data of other investigators. Indeed, in the controls they found 0.05 cell and in infected women 0.22 cell with MN per 1,000 cells. It means that in 44 samples obtained from healthy women possibly only 2 cells with MN were found, and in infected women possibly 10 cells with MN were found. 'Possibly' is being used because the total number of cells should be $0.22 \times 44 = 9.68$, which is nonsense. But if the number of cells with MN is 10, it ought to be 0.23 instead of 0.22.

All other investigators found a much higher number of cervix cells with MN in healthy women: between approximately 1.0‰ [14, 15] and 3.0–3.7‰ [2, 16]. Minimal values were reported by Aires et al. [17] (0.15‰) (3-fold higher than the value found by Safi Oz et al.) and the maximal value by Chakrabarti and Dutta [18] (4.9‰ in postmenopausal women, 98-fold higher than the value found by Safi Oz et al.). It is noteworthy that the last authors found 1.9‰ cells with MN in premenopausal women. It is important that the rates of MN in all types of epithelial cells of healthy humans are at the same level [see reviews 11, 12]. In the Turkish population, rates of MN cells in buccal mucosa are 1.95‰ [19] in adults and 1.62‰ in children [20] (in both studies Feulgen/fast green-stained cells were evaluated). Logically, the same level of MN cells should be observed in cervix cells.

The total number of cells with NB is also strange. Indeed, it should be $0.03 \times 44 = 1.32$ cells in infected women and $0.01 \times 44 = 0.44$ cells in control subjects in 44 samples. Similar strange results can be found with other nuclear anomalies in table 1 of Safi Oz et al. [1].

Another important shortcoming of the study of Safi Oz et al. [1] study is the absence of demographic data, other than the age of the women. It is well documented that many lifestyle-related factors such as smoking, alcohol consumption, use of hormonal contraceptives, artificial abortions in anamnesis [14] and menstrual status [14] can have an influence on the rates of MN in cervical cells.

The shortcomings and discrepancies mentioned should be clarified in the otherwise very interesting paper by Safi Oz et al. [1].

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