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Letter to the Editor

Comments to the paper by Benites et al. (*Genet. Mol. Res.* 2006, 5: 45-54)

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Dear Sir,

I read the research finding of Benites et al. (2006) with great interest. They showed an increased level of micronuclei (MN) and some nuclear anomalies in exfoliated buccal mucosa cells (BMC) of gas station attendants. These results are in very good agreement with data of Turkish investigators who studied the same problem (Celik et al., 2003). Brazilian researchers showed a 4.8-fold increase in the number of buccal cells with MN in exposed subjects, while it was 2.9-fold higher in Turkish gas station attendants. The nuclear anomalies - binucleates - were 1.85- and 2.7-fold more frequent in Brazilian and Turkish attendants, respectively, compared with non-exposed subjects.

Benites et al. (2006) also showed a significantly increased number of cells with MN (3.72-fold) in males from the control group compared with females. I compared the data presented by Benites et al. (2006) with the data presented by another group of Brazilian investigators - Carbonari et al. (2005) (one of the authors is listed in both papers - Martino-Roth). The number of cells with MN in the control (unexposed) groups is comparable, but Carbonari et al. (2005) found a significant, 7.2-fold increase in the number of cells with MN in females! This is really surprising because the data were obtained from subjects living in the same city - Pelotas, Brazil. Since both research groups examined 2000 Feulgen-stained cells from each participant, sample size cannot be a source of bias. The reasons for the sex-related significant difference in MN levels of subjects of the same ethnicity and living in the same city were not reported. Furthermore, Benites et al. (2006) found a 6.2-fold increase in the number of “broken egg” cells in unexposed subjects compared with the data presented by Carbonari et al. (2005) (7.96-fold in males and 1.61-fold in females). This is also very surprising! I checked the data concerning cells

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with broken egg damage in the literature. The pioneers in such studies, Tolbert et al. (1991) showed that the prevalence of this nuclear anomaly in buccal cells of individuals unexposed to any known mutagen/carcinogen subjects is very low - 0.7‰, which is close to the results of other Brazilian investigators - 0.20-0.40‰ (Ramirez and Sandanha, 2002; Cerqueira et al., 2004). The figure presented by Benites et al. (2006) - 4.35‰ is 10-fold higher than what has been reported from other investigations carried out in Brazil.

Usually, gender is considered a confounding factor for MN assays of BMC, although one of the pioneers of MN assay in exfoliated human cells, Stich (1987) showed that there were no significant differences in MN level between males versus females among healthy individuals who had not been exposed to any known mutagens. I checked some articles published in 2006 concerning this problem (Table 1). In eight of nine studies, there was no effect of gender on MN level in non-exposed subjects. In six of these studies, DNA-specific stains were used, which is very useful, because only DNA-containing bodies are marked, but not artefacts (Nersesyan, 2001; Nersesyan et al., 2006).

I do not agree with one of the conclusions of Benites et al. (2006), that the "MN assay in human exfoliated cells is one of the most sensitive methods for measuring DNA damage in human populations, because it is relatively easier to score MN compared to other methods, such as chromosome aberrations (page 52)". The MN assay does not detect DNA damage; it is only able to register clastogenic and aneugenic effects (structural and numerical chromosomal aberrations, respectively).

As for the sensitivity of MN assay in BMC, I discussed this point recently, concluding that it is less sensitive than the MN assay and counting chromosome aberrations in lymphocytes (Nersesyan, 2001). My statement is supported by Cao et al. (2002), who found that the MN assay in BMC was the least sensitive in a comparison among MN, chromosome aberrations and comet assays in evaluating lymphocytes of patients undergoing radiotherapy.

In conclusion, Benites et al. (2006) presented a set of very interesting data concerning genotoxic effects in gas station attendants. However, some points of their study should be reexamined.

Table 1. Gender differences in micronucleus frequency in exfoliated buccal cells of healthy subjects.

Country	Number of subjects, gender, age (years)	Impact of sex on micronucleus level	Stain (cells examined per subject)	Reference
Austria	8 M 22 F (33)	-	Feulgen 1000	Nersesyan et al., 2006
Brazil	20 M 10 F (27)	3.7-fold increase in male	Feulgen 2000	Benites et al., 2006
India	51 M 43 F (39)	-	Giemsa 3000	Ghosh et al., 2006
India	25 M 20 F (36)	-	DAPI 2000	Chandrasekhar et al., 2006
India	43 M 11 F (33)	-	DAPI 2000	Sailaja et al., 2006
India	107 M 58 F (33)	-	Giemsa 2000	Vuyyuri et al., 2006
Russia	26 M 26 F (6)	-	Aceto-orcein 500	Karpova et al., 2006
Russia	121 M + F (10)	-	Feulgen 1000	Aleshchenko et al., 2006
Turkey	17 M 13 F (57)	-	Feulgen 1000	Yildirim et al., 2006

M = male, F = female; - no effect.

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