

Letters to the Editor

Arsenic and Drinking Water in West Bengal

To the Editor: I read the research findings of Basu et al. (1) with great interest. However, there are some points which should be discussed.

The authors showed that the level of lymphocytes with micronuclei in control (unexposed) persons was extremely low (1.66‰). For example, in some European nations it is between 7‰ and 18‰ (2-7), and the least is in Turkish subjects—2.8‰ (8). According to the data of the Human MicroNucleus project (9), this index is 6‰ to 18‰. In Indians the micronuclei level is 7.5‰ (10) and the closest to the data presented by Basu et al.—1.6 (11). Earlier, Basu et al. (12) showed that this index in healthy persons from the same region of India was even lower—0.53‰. In both cases they studied enough cells, and this cannot be a source of bias. By the way, the level of oral cells with micronuclei is also very low compared with other data concerning healthy subjects from India (0.19-0.27; refs. 13, 14). It would be of interest to know their explanations why the number of lymphocytes with micronuclei in their studies is one of the lowest in the world!

The levels of lymphocytes and buccal cells with micronuclei in healthy persons (1.66‰ and 1.28‰, respectively) are very close. However, this ratio (lymphocytes with micronuclei / buccal cells with micronuclei) in healthy European subjects is 4.8 to 18.4 (6, 15). Even in the above-mentioned article concerning Turkish subjects (8) the ratio is 3.5. In the report of Basu et al. (1) it is only 1.29! And this needs to be explained.

I could not find in the article (1) which stain was used for epithelial cells although this information about lymphocytes is presented. This is a very important question, and I recently discussed this problem (16).

The data about the duration of living of exposed persons in the area and consuming water with arsenic are absent. For example, to study the micronuclei-inducing activity of chlorinated water on bladder cells (17) recruited people should reside at the needed area for at least 6 months. This information should be presented, which could help to calculate the cumulative dose of ingested arsenic.

On page 826 the authors discussed about higher micronuclei level in lymphocytes than in oral and bladder cells. I disagree with their explanations because it is well established that use of micronuclei in exfoliated buccal and bladder cells to detect clastogenic and aneugenic effects induced by various mutagens/carcinogens is less efficient than use of both micronuclei and chromosomal aberrations in lymphocytes (16), and there is no significant difference between them.

The authors wrote that “the three different levels of arsenic in drinking water did not affect the micronuclei proportions in the three subgroup(s) within the exposed group. This could probably be due to differences in quantity of water intake and duration of arsenic exposure in the study participants as well as interindividual variations in susceptibility to arsenic”. All three *t* in Table 3 are less than 0.05. I am not sure about the level of significance in Cochran-Armitage test, but at first glance micronuclei in lymphocytes and in bladder cells correlated with arsenic content in water significantly. And about the duration of exposure I wrote earlier, it is a shortcoming in the design of the experiment.

The authors cited an article (their ref. 43) and stated that “the high carotene content of betel leaf... make betel quid less

toxic and carcinogenic than cigarette smoking...”. However, it is well known that betel quid with and without tobacco is carcinogenic to humans—group 1 agent according to IARC expert classification (18, 19). Hence, the mentioned sentence is not correct. By the way, almost all investigators showed micronuclei-inducing activity of betel quid in oral mucosa cells; however, the data concerning micronuclei-inducing activity of cigarette smoking are contradictory (14).

Then, the authors wrote that “within the exposed group, addicted individuals exhibited significantly increased micronuclei frequency ($P < 0.01$) over nonaddicted ones in oral mucosa cells”. According to the data presented in Table 2, the difference between micronuclei levels in lymphocytes also should be significant. My calculations using Student's *t* test showed that $t=2.3$, and it should be significant at least on level of $P < 0.05$. By the way, in Table 2 all *P* values (it ought to be *p* instead of *P* in the Table 2 in my opinion) are the same ($P < 0.01$), although *U* values varied from 6.59 to 26.82!

The authors stated that “among the addicted, the increases in the micronuclei frequencies were less for all the three cell types... than in nonaddicted category”. However, they did not explain why. Moreover, on page 826, right column, the authors wrote about synergistic effect of arsenic with the betel quid chewing habit, which, of course, was a contradiction.

There are also some linguistic errors. On page 825, left column, the authors wrote “observed in unexposed lymphocyte culture”. It should be “in lymphocyte (culture) of unexposed subjects,” because the subjects were exposed, not the cultures! A little further, it is written that “...was 4.40-fold over unexposed,” which looks like an error.

In the statement of the authors that “urothelial cell micronuclei reflect damage to the bladder epithelial tissue, which occurs 1 to 3 weeks prior...” the word “urothelial” should be replaced with “epithelial”.

On page 825 it is written that micronuclei incidence in urothelial cells was found to be slightly elevated (5.02). However, 5-fold increase in the level of exfoliated cells with micronuclei is a great one for epithelial cells (14, 16)!

The authors (1) wrote that “exfoliated epithelial cells have traditionally been used for cancer screening and biomonitoring of genotoxic effects in humans (page 820)” citing the article dated 2003 although there are a lot of other sources dated much earlier. On page 826, left column, the authors wrote that “unlike the study of Warner et al. (30) in which they reported absence of significant micronuclei incidence in oral mucosa cell...”. However, the aim of the cited authors was to study micronuclei level in bladder cells.

In conclusion, very interesting data are presented with a lot of errors and shortcomings.

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In Response: With respect to the comments of Dr. Nersesyan on the article by Basu et al. (1), we summarize our replies in four points. The first point is concerned with the level of micronuclei in lymphocytes and oral mucosa cells in control/unexposed individuals; the second is with regard to the stain used for epithelial cells; the third point is related to the duration of exposure in the arsenic-exposed individuals; and the last point answers the various other questions raised by Dr. Nersesyan.

1. According to the conclusions of the Human Micronucleus Project (2), there is wide variation in the micronucleus assay because the assay is currently based on visual scoring of the slides. In the study by Fenech et al. (2), in spite of being provided slides prepared in identical manner from samples from the same culture, there was still a large extent of variation in micronuclei scores among the 34 participating laboratories (2–28 micronuclei/1,000 binucleated lymphocytes for control/untreated cultures in case of prestained slides and 1–23 micronuclei/1,000 binucleated lymphocytes for control/untreated cultures in case of lab-stained slides). This was, according to them, probably because of interscorer variation in the interpretation of scoring criteria and recognition of binucleated cells. Interpretation of the

scoring criteria ultimately depends on the subjective evaluation of each scorer. Additional lab-linked features also contribute to interlaboratory variability.

Some authors reported micronuclei frequencies lower than that reported by us in oral mucosa cells as well as lymphocytes in control individuals. Tolbert et al. (3) have estimated the frequency of cells with micronuclei to be 0.3/1,000 in buccal cells, whereas Tomanin et al. (4) have reported a frequency of 0.7 micronuclei/1,000 buccal cells. In a study on arsenic-exposed population in Mexico, the level of micronuclei in healthy unexposed individuals was observed to be 0.56/1,000 oral mucosa cells (5). The background levels of micronuclei in exfoliated buccal cells reported in the literature vary between 0.3 and 4.7 per 1,000 cells, a >10-fold variation according to Titenko-Holland et al. (6). The same level has been stated to range from 0% to 4% by Nersesyan (7) and Jen et al. (8). In a study on the assessment of cytogenetic damage in lymphocytes of dental laboratory technicians exposed to chromium, cobalt, and nickel, Burgaz et al. (9) have reported a mean frequency of 1.40 micronuclei/1,000 binucleated lymphocytes in control individuals.

Because staining with Giemsa could lead to counting of artifacts and, thus, increase the frequency of micronuclei (10), we were extremely cautious and stringent with respect to accepting a binucleated cell with a micronucleus. This is probably the reason behind the slightly low micronuclei frequencies reported in our study. Regarding data concerning healthy subjects from India, Dr. Nersesyan is probably not aware of the diversity in Indian population with respect to their origin, lifestyle, diet, and addiction habits. All these factors are supposed to affect micronuclei frequency (11). The reports by Elavarasi et al. (12) and Rajeswari et al. (13) referred by Dr. Nersesyan involved control subjects from the southern part (Chennai and Hyderabad, respectively) of India. Micronuclei level in their lymphocytes could be quite different from that found in our study subjects from the eastern part of India. The climate, lifestyle, and particularly food habits are entirely different in these two different parts of India.

The principal objective of our study was to evaluate the differences in micronuclei frequencies between the unexposed and arsenic-exposed individuals and not to validate the baseline frequency of micronuclei in our population. As all the slides were scored by the same two scorers who were highly consistent on repeat counts with good concurrence between them, variability due to subjective scoring was minimized between the arsenic-exposed and unexposed individuals. Therefore, the scoring procedure did not impact the study of differences in micronuclei frequencies between the arsenic-exposed and unexposed individuals.

2. It is true that we forgot to mention the stain used for epithelial cells. We have used Giemsa to stain both oral mucosa and urothelial cells, which is very commonly used for this purpose. Several reports exist in which Giemsa has been used to stain both the above-mentioned exfoliated epithelial cell types even after the problem discussed by Dr. Nersesyan (10). These include various genotoxicity monitoring studies in human populations (8, 13–16). Moreover, one of the important conclusions of the Human Micronucleus Project (2) regarding the effect of different staining methods on micronuclei assay scores is that the importance of staining methods is minimal.
3. It is very difficult to describe the exact duration of arsenic exposure in the exposed individuals. Although all the exposed subjects had at least 5 years of residence in their respective blocks in North 24 Parganas district and, thus, their exposure period to the contaminated drinking water was considered to be >5 years, some of the subjects had been residing in their villages for a long time and

consuming water from two or more tube wells during this period. Exactly when a particular tube well started pumping out arsenic contaminated water is unrecorded. It is a common observation that tube wells tested safe for arsenic a few months or years back may turn out to yield arsenic contaminated water in future.

- 4.
- (a) All the statistical analyses have been done by an established statistician and we confirm the correctness of the statistical tests.
 - (b) We have already stated in the manuscript that betel quid chewing with or without tobacco has been associated with oral cancer. We have never claimed that betel quid is not toxic. That is why we have considered betel quid chewing habit as an important effect modifier and we also observed significantly enhanced micronuclei frequencies in oral mucosa cells of addicted subjects over the non-addicted ones in both exposed and unexposed groups. Thus, the question raised by Dr. Nersesyan is unclear. As far as the toxicity of betel quid is concerned, we have simply reported the conclusions of a report by Babu et al. (17) that betel quid is comparatively less toxic than other forms of addiction. Moreover, we included only those individuals in our study who were mildly addicted.
 - (c) There is absolutely no error in the sentence "The increase observed for oral mucosa and urothelial cells was 4.40-fold over unexposed and 4.71-fold over unexposed, respectively, in the nonaddicted group".
 - (d) A new reference was cited with respect to the statement that epithelial cells have been traditionally used for cancer screening to provide the reader with greater number of cross-references cited in this paper in support of this statement.
 - (e) Regarding the study by Warner et al. (18), we quote a portion from their publication "The overall objective of this study was to compare the frequency of micronucleated cells in exfoliated bladder and buccal cells between a group of individuals who chronically ingest high levels of inorganic arsenic and a matched control group with little exposure to arsenic". This provides evidence in support of the conclusion that we have drawn regarding their report.

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