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Letter to the Editor

Comments on the Reports of Gandhi and Colleagues (2005) "Cytogenetic Damage in Mobile Phone Users"

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We would like to submit the following comments regarding the publications from Gandhi and Singh in International Journal of Human Genetics, 5, 259-265, 2005. Also included are some comments on the publication of Gandhi and Anita in Indian Journal of Human Genetics, 11, 99-104, 2005.

Both papers were published in the same year. It is not clear whether any of the subjects participated in both investigations. The general public is very heterogeneous in their life styles. Several changes might have occurred in the subjects belonging to different socio-economic classes during the last 5 years (when the investigators collected the information) and mobile phone use is an additional activity among many others. The general tendency in mobile phone users is to change their phones from one manufacturer to another, and also from one model to another. It is not clear whether this was the case in any of the mobile phone users. The SAR values given in both publications are apparently the published values (reported to regulatory agencies) for a particular phone; they do not necessarily correlate with the radiofrequency radiation (RFR) exposure to the individual. Also, it is not mentioned whether any of the mobile phone users had utilized a "hands free device" while using the mobile phone which could make a difference in the exposure levels. Furthermore, the RFR exposure level depends on how close the phone was held to the body of the user. Recent reviews of peer-reviewed scientific literature highlighted that a great majority of investigations did not report an induction of excess cytogenetic damage in cells exposed *in vitro* and *in vivo* to radiofrequency radiation while the authors have selectively cited the papers where an increase in such damage was observed. With respect to the experimental results, it was not indicated that all laboratory procedures were conducted simultaneously using buccal cells and blood lymphocytes from mobile phone users and control subjects. The numbers indicating the range (+/- values) in all Tables do not represent either standard deviation or standard error, and there was no description how these values were obtained.

In the paper by Gandhi and Singh (International Journal of Human Genetics., 5, 259-265, 2005) it was stated that the control group was matched with mobile phone users. However, there was a 2-fold difference between the former and the latter in the numbers of vegetarians (8/25 versus 4/25) and those belonging to upper socio-economic class (4/25 versus 8/25). The possible impact of more vegetarians in controls and 'potentially' higher fat intake in mobile phone users on the cytogenetic observations is not mentioned. There were several inconsistencies in the information provided for all subjects in Table 1. The following is one such example.

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Regarding the mobile phone use, (a) MM1 had 20 calls/day, and 23 minutes IN and 22 minutes OUT. The total duration of calls adds up to a maximum of 7.7 hours per day. This was not consistent with the daily exposure of 14 hours IN + 12.7 hours OUT calls. (b) The daily exposure of MM1 (14 hours IN and 12.7 hours OUT) will add up to a total of 26.7 hours, which is more than the total number of hours in a day. (c) Also, the daily exposure of MM1 (14 hours IN + 12.7 hours OUT) was not consistent with the total duration of 13 hours when the phone was kept on 'ON' mode. (d) It is unbelievable that MM1, described in the discussion as a 19-year-old student, "had been using mobile phone for 4.5 years with daily exposure of 12.7 hours and keeps it in 'ON' mode for 13 hours...." had time to study during those 4.5 years. (e) Considering the price of non-vegetarian food in India, it is difficult to accept that the control subjects MC8, MC9, MC10 and MC20, from middle socio-economic class, consumed 400-500 gm of non-vegetarian/day (Table 2). At the end of the discussion, it was stated "it has to be recalled that one does not have to be a cell phone user to become exposed to these radiations; exposure is there from simply living near a base station which beams radio waves or being a passenger on a crowded train full of mobile phone users". If such is the case, the control subjects are not 'real' controls (in the sense that they were also exposed to RFR).

With respect to the examination of chromosomal aberrations (CA) in blood lymphocytes cultured for 72 hours, it is well documented in scientific literature that the response of lymphocytes to mitogenic stimulation varies considerably, and during the 72 hour culture period, the ratio of lymphocytes in their first, second and third or more mitotic divisions would be approximately 20:65:15. It is also well known that cells with 'unstable aberrations' such as breaks, dicentric and ring chromosomes as well as micronuclei (MN) will get eliminated in successive mitotic divisions. Consequently, it became customary to include bromodeoxyuridine (BrdU) in the culture medium to examine the chromosomes which were stained using Fluorescence plus Giemsa technique for differential staining of chromatids which enables the identification of the lymphocytes which had gone through one, two and three or more mitotic divisions so that the evaluation of CA restricted to the lymphocytes in their first division only. The mitogen used to stimulate the lymphocytes, and whether or not BrdU was included in the culture medium was not mentioned. Further more, it is extremely rare (only in very special cases) to examine G-banded metaphase chromosomes to analyze CA since the identification of different types of aberrations in such chromosomes creates serious difficulties. The CA mentioned in the discussion refers to: (i) presence of chromosomes with centromere separation, (ii) association of acrocentric chromosomes, and (iii) triploid cells. The general reasons for these are: (i) centromere separation usually occurs as a 'technical artifact', (ii) The association of acrocentric chromosomes occurs spontaneously because of the stickiness at the end of short heterochromatic arms while synthesizing the ribosomal RNA (in the nucleoli) which render them to be associated together. Such associations are normal and are not generally considered as serious aberrations, and (iii) triploid cells mentioned as numerical aberrations could have been a consequence of culturing the lymphocytes for a relatively long time of 72 hours and they may represent anomalies during mitotic division *in vitro*. The detailed incidence of each of the above three categories in mobile phone users and in controls was not reported. It is also surprising that there were no data reporting the frequencies of 'classical' aberrations such as breaks, fragments, dicentric and ring chromosomes. Finally, the % aberrant metaphases in healthy control subjects as high as 10.66±4.59 has not been reported in the published literature.

There are several technical limitations in using buccal cells for MN investigations. (a) The evaluation does not take into consideration well known effects of cell proliferation/cell cycle. (b) The cells exhibit several confounding nuclear anomalies such as condensed chromatin, pycnosis, karyorrhexis, etc, all which may appear as MN. Nonetheless, several investigators have reported that the incidence of MN in buccal cells of healthy individuals ranged from 0.26% to 0.51% while the authors reported several-fold lower incidence of MN (0.06%) in control individuals. The 'classical' method to determine the incidence of micronucleated human blood lymphocytes has been to culture the cells for 72 hours (the last 24 hours in the presence of cytochalasin B) and to determine the incidence of MN only in cytokinesis-blocked binucleate cells.

In the paper by Gandhi and Anita (Indian

Journal of Human Genetics, 11, 99-104, 2005), regarding the MN studies, to the best of our knowledge, there was a single report by Xue et al. (1992) in scientific literature where un-stimulated human blood lymphocytes was used for MN studies, and the frequency in 98 control individuals was 0.84%. The average value in controls reported by the authors was 16.8-fold lower, i.e., 8 MN in 16,000 cells = 0.05% (and not 0.0005%). With respect to the comet assay, the authors have not mentioned whether the cells from mobile users and those from control subjects were used concurrently. Important details in the comet assay protocol (viz., durations alkaline unwinding and electrophoresis) which could influence the DNA migration length were not described. Also, the DNA migration length of silver stained comets was measured using ocular micrometer, and hence the measured length may only be an approximation. Even such approximate values were not given in detail for individual control subjects.

Our own stepwise multiple regression analysis (effect of SAR, number of years of use, daily frequency of calls, duration of IN and OUT calls, daily IN and OUT exposure, and hours when the phone was kept on 'ON' mode) using their own data indicated inconclusive, i.e., positive and negative correlations, between mobile phone use and the cytogenetic end-points examined.

For the above mentioned reasons, it is difficult to accept that mobile phone use enhances the genetoxic responses in human blood and buccal cells. The obvious inconsistencies in the general information provided for mobile phone users and our results from multiple regression analysis adds further doubts to the validity of the authors' conclusions.

REFERENCES

Gandhi G, Singh P 2005. Cytogenetic damage in mobile phone users: Preminary data. *Int. J. Hum. Genet.*, **5:** 259-265.

Gandhi G, Anita 2005. Genetic damage in mobile phone users: some preliminary findings. *Ind J Hum Genet*, **11:** 99-104.

Xue KK, Ma GJ, Wang S, Zhou P 1991. The *in vivo* micronucleus test in human capillary blood lymphocytes: methodological studies and effect of ageing. *Mutat Res* **278:** 259-264.