

Preliminary Study on the Induction of Sperm Head Abnormalities in Mice, *Mus musculus*, Exposed to Radiofrequency Radiations from Global System for Mobile Communication Base Stations

A. A. Otitolaju · I. A. Obe · O. A. Adewale ·
O. A. Otubanjo · V. O. Osunkalu

Received: 23 May 2009 / Accepted: 24 September 2009 / Published online: 9 October 2009
© Springer Science+Business Media, LLC 2009

Abstract The exposure of male mice to radiofrequency radiations from mobile phone (GSM) base stations at a workplace complex and residential quarters caused 39.78 and 46.03%, respectively, in sperm head abnormalities compared to 2.13% in control group. Statistical analysis of sperm head abnormality score showed that there was a significant ($p < 0.05$) difference in occurrence of sperm head abnormalities in test animals. The major abnormalities observed were knobbed hook, pin-head and banana-shaped sperm head. The occurrence of the sperm head abnormalities was also found to be dose dependent. The implications of the observed increase occurrence of sperm head abnormalities on the reproductive health of humans living in close proximity to GSM base stations were discussed.

Keywords Sperm head · Radiofrequency radiation · Mobile phones · Mutagens

Due to the growing use of mobile phones and the explosive growth in the multitude of base stations to meet required efficiency from the networks, there is currently an

increasing concern about the effects of electromagnetic exposure in the microwave range and radiofrequency (RF) radiation on exposed organisms and humans (Markov and Kostarakis 2007). While several studies have indicated that exposure of biological systems to low level RF radiation caused adverse biological effects, other studies have indicated that at the current exposure level to RF radiations, no adverse effects were observed (Valberg et al. 2007). The increased concern by the public about the safety and potential health effects of the multitude of cellular transmitter antennas in different neighbourhood and the fear of the unknown makes it necessary to provide answers to the questions about the safety of telecommunication base stations. At present, little can be said to be known about the effect of long term exposure that will be experienced by people living near these mobile phone base stations (Bortkiewicz et al. 2004; Abdel-Rassoul et al. 2007). Dasenbrock (2005) pointed out that if one were to be proposing new chemicals for commerce or new pharmaceuticals, the quality of available RF studies would not be acceptable for registration with responsible authorities.

As a result, a number of agencies in different countries have come up with widely varying RF safe limits or standards. For example, while the ICNIRP have set a limitation guideline of between 400–600 mV/m, other countries such as France, Italy, Switzerland and Austria have set guidelines of 200, 600, 400–500 and 600 mV/m, respectively (ICNIRP 1998; Valberg et al. 2007). This wide variation in the set guidelines from different countries is a reflection of the state of information and risk perception of the potential health effects of the RF radiations and the need to adopt the precautionary principle—“better safe than sorry” in dealing with such an important matter that is likely to have profound impact on public health. It is therefore important for more studies to be carried out to determine the acute and long

A. A. Otitolaju (✉) · I. A. Obe · O. A. Otubanjo
Department of Zoology, Faculty of Science, University of Lagos,
Akoka, Lagos, Nigeria
e-mail: bayotitolaju@yahoo.com

O. A. Adewale
Department of Physics, Faculty of Science, University of Lagos,
Akoka, Lagos, Nigeria

V. O. Osunkalu
Department of Haematology, College of Medicine,
University of Lagos, Idi-Araba, Lagos, Nigeria

term effects of the present level of RF radiation that the general populace are exposed and estimate the level of danger or otherwise that can occur.

Materials and Methods

Three locations were selected for the experiment. GSM base stations were located in two of the locations and one was a control site. For the two locations with GSM base station, one was located around a residential quarter with 2 base stations and the other located around an office block complex with only 1 base station. The third site (control station) was located away (300 m radius) from any GSM base station.

Male mice, *Mus musculus*, which served as the bioassay organisms were obtained from the Nigerian Institute of Medical Research (NIMR). The mice were fed with 40 g per day of mice feed in pellets purchased from NIMR. The mice were kept in cages for at least 14 days to acclimatise to laboratory conditions ($29 \pm 2^\circ\text{C}$ and Relative Humidity— $70 \pm 2\%$) before commencement of bioassay.

Exposure cages (LXBXH 530 cm by 350 cm by 230 cm) with perforated roof lid were used for the assay. The exposure cages were located below the GSM base stations at a distance of 1 m away. Mice were divided into three groups containing five mice each. Each group was placed in the exposure cages and placed at the designated locations.

Measurement of radiofrequency radiation from GSM base stations: Radiofrequency radiations from mobile phone base stations with frequency range from 900 to 1,800 MHz were measured with the aid of a wide spectrum Aeritalia Radiofrequency Field Strength measuring meter at distances of 0, 50, 100, 150, 200, 250 and 300 m over a 6 months period for the different locations.

Sperm head abnormality assay: Albino male mice sperm abnormality was tested according to the method of Wyrobek et al. (1983). The male mice were sacrificed by cervical dislocation after anesthetization. The epididymes were excised and minced with fine scissors in physiological saline in a petri dish. Smears were made on clean, grease-free slides after staining the cells with a mixture of normal saline (9:1) for 45 min. The slides were air-dried and coded for subsequent examination under microscope and different abnormalities were recorded. Cytological evaluation for sperm-head abnormalities was carried out using a binocular microscope at $\times 1,000$ magnification.

Appropriately test results were subjected to analysis of variance (ANOVA) between the different treatment means and the control to test the null hypothesis that there was no difference between means for the various treatments and control. Further analysis by *t*-test was carried out only where there was a significant difference at the 5%

($p < 0.05$) level of significance (taken as minimum requirement).

Results and Discussion

Analysis of sperm head abnormalities were made after 6 months of exposure to RF radiations from GSM base stations. The results of sperm head abnormality counts showed that there was a high level of abnormality in the sperm head of mice exposed to RF radiations compared to control (Table 1). The exposure of the mice to RF radiations at the workplace complex and residential complex caused 39.78 and 46.03% in sperm head abnormality score compared to 2.13% in control group. Statistical analysis (ANOVA) of the mean values of occurrence of sperm head abnormality showed that there was a significant ($p < 0.05$) difference in occurrence of sperm head abnormality in test animals. The major abnormality observed were sperm head with knobbed hook (D), pin-head (I) and banana-shaped head (J) (Fig. 1). The frequency of occurrence of the sperm head abnormality were also found to be positively correlated ($R^2 = 0.99$) (Fig. 2) to the RF radiation levels at the test locations, indicating that the frequency of occurrence of sperm head abnormalities was dose-dependent.

The elevation of frequency of sperm head abnormalities in exposed mice indicate that RF radiations may have caused damage to the pre-meiotic stages of spermatogenesis since during spermatogenesis, DNA synthesis occurs before pre-meiotic phase and no further DNA synthesis occurs throughout spermatogenesis in the cell cycle (Odeigah 1997; Otubanjo and Mosuro 2001). Several reasons have been put forward to explain the increase in the frequency of occurrence of sperm head abnormalities in organisms exposed to some chemicals. In general, damage to the sperm cell is said to occur either by physiological, cytotoxic or genetic mechanism. Odeigah (1997) reported that exposure to the chemicals could produce pituitary-hypothalamic or sex hormonal effects which in turn could

Table 1 Results of sperm head abnormality in mice exposed to radiofrequency radiations from GSM base stations

Location	Mean RF radiation (mV/m) $\pm$ SD	Mean sperm-head abnormality $\pm$ SD	% ^a
Control	59 $\pm$ 17	10 $\pm$ 5	2.13
Workplace complex	489 $\pm$ 43	350 $\pm$ 25	39.78*
Residential quarters	625 $\pm$ 25	230 $\pm$ 35	46.03*

SD standard deviation

* Significantly higher ($p < 0.05$) than control

^a The percentages are the means for groups of five mice for each location

Fig. 1 Observed shapes of normal and abnormal heads. (a) normal sperm (b) sperm with no hook (c) two tails (d) knobbed hook (e) amorphous head (f) mean bent hook (g) hook at wrong angle (h) pin-head (i) banana-shaped head after Otubanjo and Mosuro (2001)

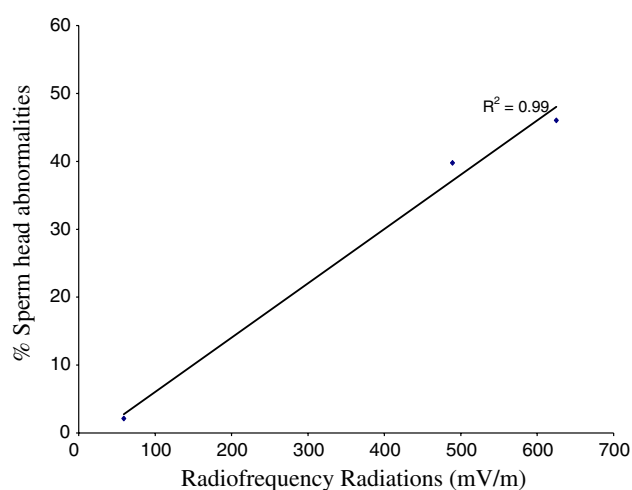
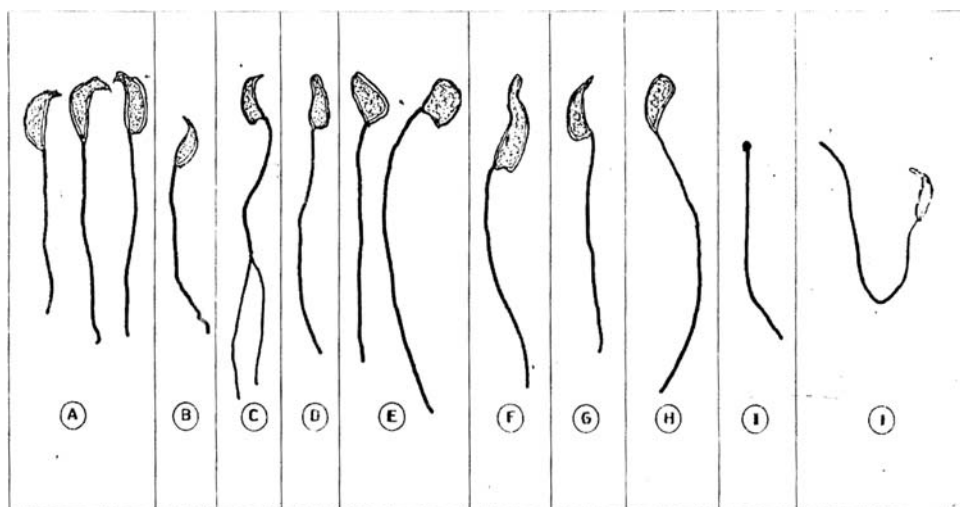


Fig. 2 Relationship between occurrence of sperm head abnormalities in mice, *Mus musculus* and level of radiofrequency radiation

affect spermatogenesis or exposure could cause abnormalities in seminal fluid resulting in functional or structural impairment of sperm. According to Beatty (1970), the development of abnormal sperm head morphology and variations in DNA content of spermatozoa are often genetically controlled. The occurrence of sperm head abnormalities have also been attributed to the chromosomal aberrations that occur during the packaging of genetic material in the sperm head or occurrence of point mutation in testicular DNA (Bruce and Heddle 1979). It may also arise as a consequence of naturally occurring level of mistakes in the spermatozoon differentiating process during spermatogenesis (Bakare et al. 2005). In this study, RF radiations acted in a similar pattern to other known chemical mutagens by increasing significantly the frequency of these mistakes during spermatogenesis.

Several studies have related large doses of radiofrequency electromagnetic radiation to genetic defects. For

example, Aitken et al. (2005) reported changes in the integrity of epididymal mitochondrial DNA in male germline. Tice et al. (2002) reported that exposure to RF radiations cause an increased micronuclei formations in human blood cells. Mashevich et al. (2003) and Diem et al. (2005) stated that RF radiations caused increased chromosomal instability and DNA breakage. While Pacini et al. (2002) reported alterations in gene expression, proliferation and morphology of human skin fibroblast. The observation in this study of an increase in frequency of occurrence of sperm head abnormalities in exposed mice therefore adds to the growing evidence within the scientific community of the potential reproductive health effects of the constant exposure to RF radiations especially from GSM base stations which currently dots the landscape of many developing countries including Nigeria. The real environmental concern in many of these developing countries is the non-discriminatory manner in which these base stations are sited in close proximity to residential homes, offices, hospitals and schools therefore increasing exposure level. This high level of exposure may ultimately cause problem to the general populace in the long term since there is really no conclusive evidence that RF radiations are safe. Although there is inherent difficulty in extrapolating results obtained in mice to humans, the results from this study, notwithstanding, gives an indication of potential public health risks that can be associated with exposure to RF radiations and implies that regulatory agencies in these countries ought to adopt the precautionary principle—“better safe than sorry” in dealing with matters related to human exposure to RF radiations.

Acknowledgments This research is sponsored by the University of Lagos Central Research Committee (CRC) grant No. 2007/18. Ethics: The experiments carried out involved the utilization of whole and live mice. However, aiming for the protection and welfare of animals, studies were conducted in accordance with the Nigerian and

University of Lagos Ethics Committee guidelines for experiment with whole animals.

References

- Abdel-Rassoul G, Abou El-Fateh O, Abou Salem M, Michael A, Farahat F, Batanouny M, Salem E (2007) Neurobehavioral effects among inhabitants around mobile phone base stations. *Neuro Toxicol* 28:434–440
- Aitken R, Bennetts L, Sawyer D, Wiklendt A, King B (2005) Impact of radiofrequency electromagnetic radiation on DNA integrity in the male germline. *Int J Androl* 28:171–179
- Bakare AA, Mosuro AA, Osibanjo O (2005) An in vivo evaluation of induction of abnormal sperm morphology in mice by landfill leachates. *Mutat Res* 582:28–34
- Beatty RA (1970) The genetics of the mammalian gamete. *Biol Rev* 45:2–119
- Bortkiewicz A, Zmyslony M, Szykowska A, Gadzicka E (2004) Subjective symptoms reported by people living in the vicinity of cellular phone base stations. *Rev Med Pr* 55:345–351
- Bruce WR, Heddle JA (1979) The mutagenic activity of 61 agents as determined by the micronucleus, Salmonella and sperm abnormality assays. *Canada J Genetic Cytol* 21:319–334
- Dasenbrock C (2005) Animal carcinogenicity studies on radiofrequency fields related to mobile phones and base stations. *Toxicol Appl Pharmacol* 207(2):342–346
- Diem E, Schwarz C, Adlkofer F, Jahn O, Rudiger H (2005) Non-thermal DNA breakage by mobile-phone radiation (1800 MHz) in human fibroblasts and in transformed GFSH-R17 rat granulose cells in vitro. *Mutat Res* 583:178–183
- ICNIRP–International Commission on Non-Ionising Radiation Protection (1998) Guidelines for limiting exposure to time-varying magnetic and electromagnetic fields up to 300 GHz. *Health Phys* 74:494–522
- Markov M, Kostarakis P (2007) Biological effects of electromagnetic fields. *Environmentalist* 27:385
- Mashevich M, Folkman D, Kesar A, Barbul A, Korenstein R, Jerby E (2003) Exposure of human peripheral blood lymphocytes to electromagnetic fields associated with cellular phones leads to chromosomal instability. *Bioelectromagnetics* 24:82–90
- Odeigah PGC (1997) Sperm-head abnormalities and dominant lethal effects of formaldehyde in albino rats. *Mutat Res* 389:141–148
- Otubanjo OA, Mosuro AA (2001) An in vivo evaluation of induction of abnormal sperm morphology by some anthelmintic drugs in mice. *Mutat Res* 497:131–138
- Pacini S, Ruggiero M, Sardi I, Aterini S, Gulisano F, Gulisano M (2002) Exposure to global system for mobile communication (GSM) cellular phone radiofrequency alters gene expression, proliferation and morphology of human skin fibroblasts. *Oncol Res* 13:19–24
- Tice RR, Hook GG, Donner M, McRee DI, Guy AW (2002) Genotoxicity of radiofrequency signals. I. Investigation of DNA damage and micronuclei induction in cultured human blood cells. *Bioelectromagnetics* 23:113–126
- Valberg PA, van Deventer E, Repacholi MH (2007) Workgroup report: base stations and wireless networks–radiofrequency (RF) exposures and health consequences. *Environ Health Perspect* 115(3):416–424
- Wyrobek AJ, Gordon LA, Burkhart JG, Francis MW, Kapp Jnr RW, Letz G, Mallin HG, Topham JC, Whorton M (1983) An evaluation of the mouse sperm morphology test and other sperm tests in non-human mammals. A report of United States EPA Gene-Tox program. *Mutat Res* 115:1–72