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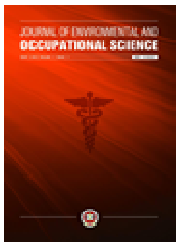

Review Article

Haematological effects of radiofrequency radiation from GSM base stations on four successive generations ($F_1 - F_4$) of albino mice, *Mus Musculus*

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Abstract

Aim: The aim of the study is to evaluate the haematological effects of radiofrequency (RF) radiation from Global Systems for Mobile Communication (GSM) base stations on four successive generations of albino mice *Mus musculus* were evaluated.

Methods: Each generation of mice were exposed to the RF radiation from birth till they reproduced and blood samples collected. After the mice had given birth to the young ones and weaned off, the parent group was sacrificed and 500µl of blood was obtained. Estimation of hemoglobin concentration, white blood cell count, platelet count, red blood cell count, lymphocyte, pack cell volume (PCV) and red cell indices were carried out using electronic coulter counter.

Results: The level of radiofrequency radiation around the base station was found to range between 0.6129V/m to 1.695V/m. In the successive generations of mice, a pattern of pancytosis was observed and significant increases were observed in the Packed Cell Volume, White Blood Cell Count, Platelet Count and Red Blood Cell Count.

Conclusion: In the successive generations of exposed mice, a pattern of pancytosis was observed, as well as, an increase in the haemopoetic process which caused significant effects on bone marrow stem cell proliferation of differentiation. The effects of chronic exposure to radiofrequency radiations on peripheral blood parameters is found to be sufficient to apply the precautionary principle to discourage the indiscriminate location of GSM base stations in areas where prolonged exposure to radiofrequency radiations are likely to occur.

INTRODUCTION

A recent report by the committee on the environment for the Council of Europe, recommends that member states should take all reasonable measures to reduce exposure to electromagnetic fields, especially to radio frequencies from mobile phones, and reconsider the scientific basis for the present electromagnetic fields exposure standards set by the International Commission on Non-Ionising Radiation Protection (ICNIRP), which have serious limitations and apply "as low as reasonably achievable" (ALARA) principles, covering both thermal effects and the athermic or biological effects of electromagnetic emissions or radiation [1]. Among the bases for the declaration by the committee is the lack of clarity and sufficient data to enable proper determination of long term effects of electromagnetic radiations from mobile phones and GSM base stations.

Although, several studies have indicated that exposure of biological systems to low level RF radiation caused adverse biological effects, there are other reports which have indicated that at the current exposure level to RF radiations, no adverse effects were observed [2]. For instance, the interaction of non- ionizing radiations with biological systems have been reported to cause

perturbations in biochemical reactions, increase reaction rates, current flow and the integrity of cell membranes [3]. It has also been reported to promote the production of free radicals [4], cause hemolysis [5], increase occurrence of sperm head abnormalities [6] and produce DNA single strand breaks [7]. Conversely, other studies have indicated that at the current exposure level, no effects were observed [8; 9]; and indeed a few studies even seem to suggest that the exposure to the low level RF radiation seem to have protective effects [10]. Therefore, it is generally agreed that information on the potential effects of RF radiations especially effects that can arise as a result of long term exposure to non-ionizing radiations from mobile phone base stations [11;12). As a result, more studies on effects of RF radiations on a wide variety of parameters spanning over several generations in exposed organisms will be required in order to elucidate the potential long term effects of these RF devices.

Studies on the haematological effects of radiofrequency radiations will therefore serve as a useful general indicator of the potential of RF radiations to cause adverse effects on exposed organisms. This is because the blood is a pathophysiological reflector of the whole body and, therefore, blood parameters are important in diagnosing the structural and functional status of organisms exposed to toxicants [13; 14]. On the basis of the above, the objectives of this study are to determine the haematological effects of RF radiation from GSM base stations on four successive generations ($F_1 – F_4$) of albino mice, *Mus musculus*.

MATERIALS AND METHODS

The radiofrequency radiation was measured at the GSM base station at different distances of 0, 50, 100, 150, 200, 250 and 300 m which was used in a period of eight months. 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 Radiofrequency Field Strength Meter (Manufacturer–Aeritalia; S/N -480836).

Female and Male mice were obtained from National Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria, to acclimatize to laboratory conditions (29 ± 2^0C and Relative Humidity 70 ± 2%) before commencement of bioassay. The mice were divided into two groups and put in exposure cages (LXBXH 530 cm by 350 cm by 230 cm) with perforated roof lid for the respective bioassays. The first groups was exposed to radiofrequency radiations (1.4 – 1.7V/m) from a telecommunication base station located in an office area inside University of Lagos campus (DLI BS) at a distance that is approximately 250m away from the telecommunication base station. The second group was placed at a site which had low level of radiation (0.05 – 0.2V/m) (control). Both groups were fed with pellets (40g per day) and allowed to breed at the exposure site until four successive generations. Each generation of mice was removed from the parent cage after about 30 days when they had been weaned off their parent milk and placed in a separate exposure cage where they were fed on mice pellets and allowed to breed. The experiment including breeding lasted for 349days at both exposure and control sites. Each generation of mice were exposed to the RF radiation from birth till they reproduced and this span over a period of 60 – 90days.

In the initial parent generation group, 7 females and 3 males per exposure cage in 3 replicates were used for the experiment, giving a total of 21 females and 9 males. For the $F_1 – F_4$ generations, 7 females and 3 males chosen randomly from the young ones given birth to by the successive parent group were placed in each exposure cage. A total of four replicates were prepared giving a total of 28 females and 12 males per generation.

After the mice had given birth to the young ones and weaned off, the parent group was sacrificed by cervical dislocation after anesthetization. 500µl of blood was obtained from the eyes of mice using heparinized hematocrite bottle and stored in an EDTA anticoagulant bottle. Estimation of hemoglobin concentration, white blood cell count, platelet count, red blood cell count, lymphocyte, pack cell volume (PCV) and red cell indices were carried out using electronic coulter counter.

Data sets obtained were subjected to analysis of variance (ANOVA) and unpaired T-test between the different treatment ($F_1 – F_4$ generations) means and the control (Parent generation) to test the null hypothesis that there was no difference between means for the various treatments and control. Further analysis of associations by chi-square was carried out where there was a significant difference at the 5% ($P< 0.05$) level of significance (taken as minimum requirement).

RESULTS

Measurement of radiofrequency (RF) radiation

The results of the radiofrequency measurements are given in Figure 1. The level of RF radiation around the base station was found to range from 0.6V/m to 1.7V/m and this level is significantly higher than the RF radiation in the control site (devoid of base station within a 300m radius) which ranges from 0.05V/m to 0.37V/m.

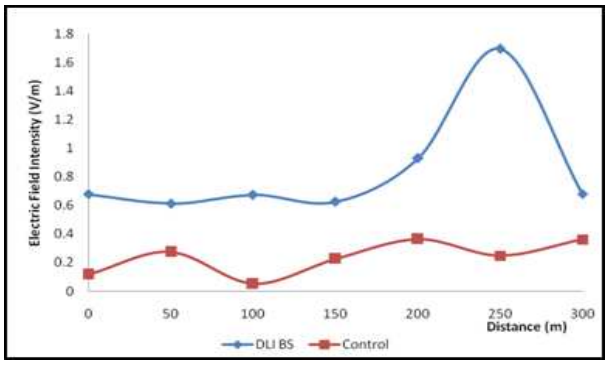

Figure 1. Level of Radiofrequency radiations around the GSM base station and a control area without base Station within a 300m radius.

Haematological studies

Red Blood Cell (RBC) Count: This is used to indicate the total mass of Red Cell per liter of whole blood. The mean value of red blood cell count for control mice was given as 8.36 x 10^6µL. The mean value of red blood cell count for first generation was given as 72.79 x 10^6µL. The mean value for second generation was 9.698 x 10^6µL, for third generation the mean value was 9.813 x 10^6µ L while the mean value for the fourth generation was 9.973 x 10^6 µ (Table 1).

Table 1: Comparison of mean values for Red Blood Cell Count (RBC) (x 10^6µL)

MICE GROUP	MEAN± SEM	T – TEST (P- VALUES)
Control	8.360 ± 0.6494	
First generation	72.79 ± 21.24 *	0.0230
Second generation	9.698 ± 0.5215	0.1594
Third generation	9.813 ± 0.6523	0.1656
Fourth generation	9.973 ±0.5809	0.1362

* Statistically significant (P<0.05) compared to control

White Blood Cell (WBC) Count: This is a reflection of the total value of lymphocytes and granulocytes per liter of mice whole blood. The mean value for white blood cell count for control mice was given as 8.85 x 10^3µL. The mean value of white blood cell count for first generation was 15.93x 10^3µL. The mean value for second generation was 35 x 10^3µL, for third generation the mean value was 35 x 10^3µL and the mean value for fourth generation was 15.73 x 10^3µL (Table 2).

Table 2: Comparison of mean values for White Blood Cell (WBC) (x 10^3µL)

MICE GROUP	MEAN ±SEM	T – TEST (P-VALUES)
Control	8.85 ± 1.20	
First generation	15.93 ± 6.593	0.0193
Second generation	35 ***	0.0001
Third generation	35 ***	0.0001
Fourth generation	15.73 ± 9.6364 *	0.0104

*Statistically significant (P<0.05) compared to control

***Statistically significant (P<0.0001) compared to control

Platelet Count (PLT): An average of the total number of platelet per liter of whole blood was evaluated. This showed that the mean platelet count for the control mice was 539.5 x 10^3µL. The mean value for first generation was 633 x 10^3µL. The mean value for second generation was 1032 x 10^3µ L, the mean value for third generation was 887.8 x 10^3µL and the mean value for fourth generation was 909.3 x 10^3µL (Table 3).

Table 3. Comparison of mean values for Platelet count (PLT) (x 10^3µL)

MICE GROUP	MEAN ± SEM	T- TEST (P-VALUES)
Control	539.5 ± 79.92	
First generation	633± 98.23	0.04882
Second generation	1032 ± 241.1	0.1004
Third generation	887 ± 171.6	0.1155
Fourth generation	909.3 ± 52.25 *	0.0164

*Statistically significant (P<0.05) compared to control

Red Cell Indices

Mean Cell Volume (MCV): This indicates the average volume of single red cell and also measures foliate and vitamin B 12 deficiency. The Mean Cell Volume for control mice was 52.78fL. The Mean Cell Volume for first generation mice was 58.35fL. The value for second generation was 57.95fL, third generation was 61.48fL while that of fourth generation was 55.2fL (Table 4).

Table 4. Comparison of mean values for Mean Cell Volume (MCV) (fL)

MICE GROUP	MEAN ± SEM	T – TEST (P- VALUES)
Control	52.78 ±1.592	
First generation	58.35 ± 1.007 *	0.0253
Second generation	57.95 ± 0.8251 *	0.0279
Third generation	61.48 ± 2.289 *	0.0206
Fourth generation	55.20 ± 0.6658	0.2719

*Statistically significant (P<0.05) compared to control

Mean Cell Haemoglobin (MCH): This refers to the quantity of haemoglobin per red cell in whole blood. The Mean Cell Haemoglobin for control mice was given as 15.55pg . The Mean Cell haemoglobin for the first generation mice was 16.68pg. Second generation Mean Cell haemoglobin was 15.18pg, third generation mice was 16.85pg and fourth generation was 11.15pg (Table 5).

Table 5. Comparison of mean values for Mean Cell Haemoglobin (MCH) (pg)

MICE GROUP	MEAN ± SEM	T – TEST (P- VALUES)
Control	15.55 ± 0.3096	
First generation	16.68 ± 0.7983	0.2369
Second generation	15.18 ± 0.8260	0.6856
Third generation	16.85 ± 0.9394	0.2367
Fourth generation	11.15 ± 3.723	0.2835

Mean Cell Haemoglobin Concentration (MCHC): This indicates the value of haemoglobin in a packed cell. It is also indicative of iron (Fe) deficiency. The Mean Cell Haemoglobin Concentration for control mice was 29.6g/dL. The mean value for first generation mice was 28.55g/dL. Second generation was 26.15 g/dL, third generation was 27.38 g/dL and fourth generation was 20.20 g/dL (Table 6).

Table 6. Comparison of mean values for Mean Cell Haemoglobin Concentration (MCHC) (g/dL)

MICE GROUP	MEAN ± SEM	T- TEST (P- VALUES)
Control	29.60 ± 1.372	
First generation	28.55 ± 1.043	0.5646
Second generation	26.15 ± 1.380	0.1266
Third generation	27.38 ±0.6290	0.1908
Fourth generation	20.20 ±6.738	0.2206

Packed cell Volume (PCV): This refers to the propulsion of red cell per liter of whole blood. The PCV value for control mice was given as 43.95%. The mean value for first generation mice was given as 56.33%, for second generation the mean value was 56.3%, while third generation was 59.88% and fourth generation was 55% (Table 7).

Table 7. Comparison of mean values for Packed Cell Volume (PCV) (%)

MICE GROUP	MEAN ± SEM	T-TEST (P-VALUES)
Control	43.95 ± 2.977	
First generation	56.33 ± 3.350 *	0.0328
Second generation	56.30 ± 3.735 *	0.0414
Third generation	59.83 ±1.875 **	0.0041
Fourth generation	55.00 ± 2.754 *	0.0468

*Statistically significant (P<0.05) compared to control

**Statistically significant (P<0.001) compared to control

DISCUSSION

The maximum level of radiofrequency radiation based on field strength was 1.695V/m and this was detected at 250m from the base station at DLI, this could be as a result of the topography of the land. The RF radiation around the base station at DLI was found to be significantly higher than the RF radiation in the control station which was devoid of mast. The values of the RF radiation detected at studied base station are several folds lower than the 40-60 V/m safe limit range by the International Commission on Non ionizing Radiation Protection [15]. It is however important to note that at present little can be said to be known about the effect

of long term exposure that would be experienced by people living near these mobile phone base stations [11; 13]. This uncertainty has led to the setting of differential guidelines by regulators in different countries. For example, while the ICNIRP set a limitation guideline of between 40-60V/m, other countries such as France, Italy, Switzerland and Austria have set guidelines of 2V/m, 6V/m, 4-5V/m and 6V/m respectively. 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 effect of the RF radiations.

The results of the haematological studies indicate varied effects of RF radiations on the different haematological parameters. The Packed Cell Volume (PCV) of control mice (43.95%) showed a statistically significant pattern of increase when compared with the PCV of exposed generations (first generation, second generation, fourth generation) (56.33%, 56.3%, 55%, $P<0.05$ respectively) and third generation (59.83%, $P<0.001$). The mean Red Cell Count for the exposed second, third and fourth generations mice were observed to have increased (9.698 x $10^6\mu L$, 9.813 X $10^6\mu L$ and 9.973 x $10^6\mu L$ respectively) when compared to the mean Red Cell Count of 8.36 x $10^6\mu L$ for the control mice and having a high statistically significant level for the exposed first generation (72.79 x $10^6\mu L$, $P<0.05$). These findings indicate an absolute increase in Red cell mass rather than a dehydrative process as earlier reported by Villa *et al*., [16]. There was an observed increase in the overall white cell count of the first, second, third and fourth generations (15.93 x $10^3\mu L$, 35 x $10^3\mu L$, 35 x $10^3\mu L$, 35 x $10^3\mu L$, 15.73 x $10^3\mu L$ respectively) compared to the control value (8.85 x $10^3\mu L$). The increase was statistically significant ($P<0.05$) for first and fourth generations, then ($P<0.0001$) for second and third generations. The white blood cell count for second and third generations mice were above the standard value, the sysmex haematological machine could read (that is > 35 x $10^3\mu L$), this value was used as a representative value in order to determine how statistically significant they were compared to the control. This increase may be related to the induction of a protective mechanism in the exposed mice to the effect of the RF radiation and other activities around the GSM base stations. Bastide *et al,* [17] reported from their study of mice exposed to GSM radiation and there was 50% decrease in immunoglobulin levels and a further 50% decrease in serum levels of corticosterone. Hence, it can be deduced that exposure to radiofrequency waves induces stress in exposed animals and increase susceptibility to infections, which may also lead to the synthesis of abnormal levels of white blood cell. Several studies including [Pocock *et al*., [18]; Hoffman *et* al., [19] and Jee *et al* [20] have identified the white blood cell count as an integrated indicator of inflammatory stimuli on both acute and chronic time frames. It is elevated acutely by infection and other stresses or toxic exposures.

There was an observed increase in platelet count from the control mice value of 539.5 x $10^3\mu L$ when compared to the first, second, third and fourth generations of exposed mice with values of 633 x $10^3\mu L$, 1032 x $10^3\mu L$, 887.8 x $10^3\mu L$ and 909.3 x $10^3\mu L$ respectively. The increase was statistically significant in the fourth generation mice ($P<0.05$), thus establishing a pattern of pancytosis (a general increase in all cell lines). Red Cell Indices are haematological pointers to the utilization or consumption of iron (MCH and MCHC) and folate (MCV). The Mean Cell Volume (MCV) of the control mice is 52.78 fL which is statistically significant compared to those from first generation (58.35fL), second generation (57.95fL) and third generation (61.48fL) ($P<0.05$ respectively). The Fourth generation (55.2fL) was not statistically significant ($P>0.05$). The Mean Cell Haemoglobin (MCH) of the control mice is 15.55pg which is not statistically significant ($P>0.05$) from the values of first, second, third and fourth generations (16.68pg, 15.18pg, 16.85pg and 11.15pg respectively). The Mean Cell Haemoglobin Concentration (MCHC) of the control mice is 29.6 g/dL which is also not statistically significant from the values of the first generation (28.55 g/dL), second generation (26.15g/dL), third generation (27.38g/dL) and fourth generation (11.15g/dL) ($P>0.05$ respectively).

Increase in haematological parameters observed in this study, strongly indicates an increase in haemopoetic process possibly induced by electromagnetic effects on mice bone marrow stem cell proliferation and differentiation. There was also evidence of iron lack due to increased haemopoesis which lead to microcytosis. However, clinical anaemia was not observed. This is contrary to the report of Manisha and Baile [21] who reported anaemia in the exposed rat. This observed differences in the haematological parameters of mice exposed to EMF radiation in various studies done, might not be unconnected to the strength of the electromagnetic fields and the total duration of exposure as uncontrolled haemopoesis might eventually lead to anaemia and bone marrow atrophy or fibrosis.

Conclusion

In the successive generations of exposed mice, a pattern of pancytosis was observed and significant increases were observed in the Packed Cell Volume, White Blood Cell Count, Platelet Count and Red Blood Cell Count. Increase in the haemopoetic process induced by radio frequency radiation caused significant

effects on bone marrow stem cell proliferation of differentiation and there was an evidence of iron which led to microcytosis. The effects of chronic exposure to radiofrequency radiations on peripheral blood parameters is found to be sufficient to apply the precautionary principle in order to discourage the indiscriminate location of GSM base stations in areas where prolonged exposure to high level of radiofrequency radiations are likely to occur.

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

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