Research Article

10 GHZ Microwave Exposure during Pregnancy Causes Postnatal Changes in Swiss Albino Mice

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Accepted on: 20-08-2016; **Finalized on:** 31-10-2016.

ABSTRACT

10 GHz microwave (MW) exposure during pregnancy from different gestation days (0.25/11.25) till term and its effects on neonates and 3 week aged mice were evaluated. Pregnant females from 0.25 and 11.25 day of gestation were selected and divided into 2 groups. Sham exposed- Mice were placed in plexiglass cage with system off for 2 hours/day till term. MW exposed- Mice were exposed to 10 GHz for 2 hours/day till term at power density 0.25 mW/cm^2 and SAR 0.1790 W/kg. After delivery 50% neonates were studied for various morphometrical and hematological changes. Remaining 50 % were allowed to grow till three weeks of age for follow-up study. Morphometric analysis revealed non-significant changes in litter size and average crown rump length in newborns of exposed and unexposed females at both gestational days (0.25/11.25) with significant decrease in bodyweights in MW exposed groups. Loss in bodyweights was greater in mice whose mothers were exposed throughout the term. Hematological analysis revealed architectural disturbances in erythrocytes of neonates and 3 week old mice born to 10-GHz exposed females. 10 GHz MW exposure during pregnancy affects the body weights and blood of neonates and 3 week old mice dependent on duration of exposure. The follow up study of the neonates at three weeks of age showed alterations in blood and spleen. Exposure during pregnancy has long term effects as evident by follow up study at three weeks which is dependent upon the duration of exposure during pregnancy.

Keywords: Neonates, erythrocytes, electromagnetic field, litter size, body weight, crown rump length.

INTRODUCTION

The exposure of the developing fetus and children to electromagnetic field (EMF) including both radiofrequency radiation (RF) used in new wireless technologies, and to extremely low frequency (ELF) or power frequency fields has raised public health concern because of the possible effects (cancer, neurological effects, developmental disability effects, etc) from the long term exposure to low intensity, environmental level fields in daily life. Several studies reported adverse health effects at levels below safety standards.[1]The evidence to date suggests that special attention should be devoted to the protection of embryos, fetuses and newborns that can be exposed to many diverse frequencies and intensities of EMF throughout their lifetime, where the health and wellness consequences on these subjects are still scarcely explored. However, some of the studies have suggested that ELF-EMF disturb the development of non mammalian embryos leading to induction of structural anomalies (Farrell *et al.* 1997). Mammalian studies have reported increased risk of miscarriage caused by ELF-EMF exposure during pregnancy in women, changes in uterus and ovary in rats, congenital deformities and developmental delay in the offspring of mice, increased height of mice fallopian tube epithelial cells, reduced fertility in female rats and development of bones in rats. [2, 3] Studies have reported exposure to cell phone radiation during early gestation at Specific Absorption Rate (SAR) of 0. 5 mW/cm^2 (milli watt per centimeter square) affected kidney development in rats.[4] In mammals results have been inconsistent; some studies reported no or only slight effects on fetuses that included slight limb anomalies and body weight reduction.[5] But studies reported that prenatal exposure to EMF induces adverse effects such as growth retardation, delayed puberty in newborn rats.[6] Moreover, only 48 hour (hrs) exposure to ELF-EMF in pregnant rats increases incidence of non vital fetuses, neonates or infants.[7] More recently EMF has been reported to affect a wide range of other basic cellular functions. At cellular level many studies have shown that both prenatal and post natal exposure to ELF-EMF induces noxious effects in mammals.[8] Epidemiologically, the effects of these fields on embryogenesis have suggested a possible relationship between EMF and adverse pregnancy outcomes in humans and animals. The 10 Gigahertz (GHz) band is the easiest microwave bands to get on primarily because of its proximity to frequencies heavily used by different radars and the resulting equipment availability. Its increased usage in occupational environment has caused potential threat to human health, resulting in growing public concern. [9, 10] This has attracted a great deal of attention. The present study has therefore undertaken to study the effects on neonates and on attaining 3 weeks of age after 10 GHz microwave (MW) exposure to pregnant females from different days of gestation (0.25/11.25).

MATERIALS AND METHODS

Experimental Model (Female Swiss Albino Mice)

Female pregnant mice from 0.25 and 11.25 days of gestation were selected for the present study. These animals were maintained in the animal house as an inbred colony as per the norms established by Institutional Animal Ethical Committee (IAEC). They were housed in clean polypropylene cages and maintained under controlled conditions of temperature (25 ±1.5ºC) , 12 hours light and 12 hours dark (12L: 12D). They were maintained on standard normal diet obtained from Hindustan Lever, Delhi, India and water *ad libitum.*

Determination of Pregnancy

Females and males were housed in the cages in the ratio 3:1 for determination of pregnancy. Early in the morning the females were checked for vaginal plugs. The day at which vaginal plug appeared was considered as 0 day of pregnancy. The females under consideration were separated. Assuming that the mating , in mice took place after midnight and exposure to radiation was done between 9 to 11 am, the embryological ages at the time of irradiation , therefore, corresponded approx to days $1/4^{th}$(0.25) and 11 $^{1/4th}$ (11.25) post conception . These embryological ages represent developmental stages corresponding to the period of organogenesis (day 11.25).

10 GHz Exposure System, Exposure Conditions and Dosimetry

Two mice were housed at a time in a rectangular partitioned cage made of plexiglass which was well ventilated with holes of 1 centimeter (cm) diameter. The dimensions of the cage (4.5×9×9cm) were such that animals were comfortably placed, though they could not move. The horn antenna was kept in H (Magnetic field) plane configuration (Sharma et al. 2014). Therefore electric field was perpendicular to the ground surface. Field was almost uniform because the dimensions of the cage were of the order of wavelength. At near field distance from the horn antenna, power density measured was 0.25 mW/cm^2 (milliwatt per centimeter square) which was maximum. Everyday, the cage with mice was placed in the same position facing the horn antenna. The mice were exposed with 10 GHz MW radiation source through the antenna for 2 hrs/ day for 30 days as shown in Figure 1. The whole microwave exposure system was procured from Wavetech, Faridabad, Haryana, India. The emitted power of microwaves was measured by a power meter which is a peak sensitive device (RF power sensors 6900 series and infra red (IFR) 6960 B RF power meter; made of Aeroflex Inc., Wichita, Kansas, USA). Every day the cage was placed in the same position in front of horn antenna. A similar experiment was performed with sham exposed animals without energizing the system. The power density at the cage location was 0.25 mW/cm^2 and the SAR was calculated as 0.1790 W/Kg (watt per kilogram) following the work of Durney.[11]

Figure 1: Sketch of the experimental arrangement of 10 GHz microwave source

Preparation of Blood Smear

Blood cells were studied in smears prepared by spreading a drop of blood thinly over a clean and sterilized microscopic slide with the help of another slide moved over the first at the angle of 45^0 after discarding first drop of blood. These blood films were air-dried and fixed in absolute methanol for 15 minutes by dipping the film briefly in a coupling jar containing absolute methanol. After fixation the slides were removed and air-dried and blood smears stained with freshly made giemsa stain. The stain was diluted with water buffered to pH 6.8 or 7.0 (1:9) and buffer respectively. The slides were washed by briefly dipping the slide in and out of a coupling jar containing buffered water and air dried again for taking observations.

Table 1: Experimental protocol

Gestation day	Exposure duration (2 hrs/day)	Total duration of exposure	Sham exposed	Microwave exposed	Blood collection
0.25	Till term	20 days	System off	10 GHz 2 hrs/day	After birth and at 3 weeks of age
11.25	Till term	8 days	System off	10 GHz 2 hrs/day	After birth and at 3 weeks of age

Table 2: Variations in the average number of litters, crown rump length and bodyweight of mice (1 day and 3 week old) after 10 GHz MW exposure of pregnant females from 0.25 day and 11.25 day of gestation till term.

Treatments	Gestation day	No. of litters	Crown rump length (cm)	Body weight after birth (grams)	Body weight at 3 weeks of age (grams)	Spleen weight at 3 weeks of age (mg)
Sham exposed	0.25 day	4.33±0.21	2.87±0.01	1.90±0.05	10.31±20.12	50.56±0.38
	11.25 day	4.33±0.21	2.87±0.01	1.90±0.02	10.26±0.11	50.68±0.56
MW exposed	0.25 day	4.33±0.21^n	2.86±0.01^n	1.01±0.03*	9.81 ±0.20*	45.99±0.75*
	11.25 day	4.33±0.21^n	2.87±0.01^n	1.05±0.04*	9.16±0.05*	48.2±0.45*

Each value represents Mean ± SEM; Statistical comparison: Sham Vs MW exposed; Significance levels: *P < 0.001; n=non significant differences

Experimental Protocol

Pregnant female mice from 0.25/11.25 days of gestation were assigned to each of the following groups: Sham exposed (system off) and microwave exposed (10 GHz 2 hours/day till term). After delivery 50 % newborns were immediately and remaining newborns were left to grow until 3 weeks of age for the follow up study. After that blood was studied for hematological parameters and spleen were studied for biochemical parameters (Table 1).

1. **Morphometric Studies:** Body weight, Crown Rump Length (CRL) and litter size
2. **Biochemical studies:** LPO and GSH in spleen
3. **Histopathological Studies:** Blood

Statistical Analysis

Statistical analysis was done using student's t-test. Results were statistically significant at $P < 0.001$

RESULTS

Morphometric Studies

No external malformations were noticed in the newborns examined in the present investigation. The litter size recorded for the animals used in the present study was 4.33. Non-significant difference was noted in litter size of pregnant females from gestation day 0.25/11.25 in both exposed and unexposed groups. Average crown rump length measured in newborns of sham and microwave exposed exhibited non-significant difference irrespective of gestation day (0.25/11.25) from which the exposure was made. Body weights of the animals exposed prenatally were compared to the same of their coeval controls. The results of the present investigation showed that the average bodyweights of the newborns significantly decreased ($p<0.001$) in MW exposed groups .It was observed that exposure from early gestation day (0.25 day) resulted in greater loss of weight compared to 11.25 day of gestation. Follow up study conducted at 3 weeks also showed similar pattern i.e. greater is the duration of exposure more is loss in weight.

Spleen biochemistry

Follow-up study of mice at three weeks of age after completion of exposure to pregnant females from different gestation days showed significant increase in lipid per oxidation (LPO) and significant decrease in GSH levels compared to sham exposed (Table 3).

In neonate studies immediately after birth exposure to MW from different gestational days (0.25 and 11.25) resulted in poikilocytosis (Po) of erythrocytes which is a possible indicator of blood cancer. Most of the red blood cells (RBCs) became hypochromic, microcytes were also seen (fig 5). Overlapping of erythrocytes was seen in neonates whose mothers were exposed with radiation from 0.25 day of gestation (Fig. 3). Whereas, neonates of sham exposed females showed original disc shaped form of RBCs having regular amount of hemoglobin.

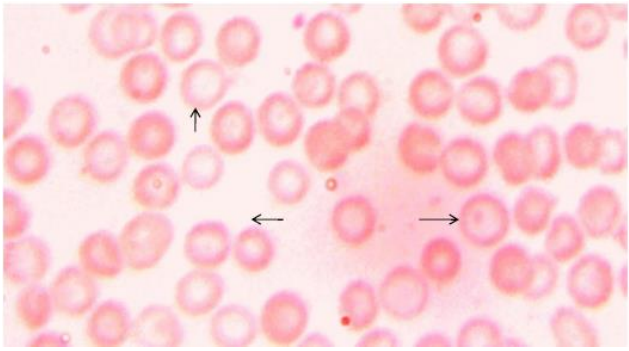

Figure 2: Blood smear of newborns of sham exposed pregnant females from 0.25 day of gestation showing round and discoid normocytes with no overlapping. (Geimsa stain; 100 X (oil immersion) x magnification).

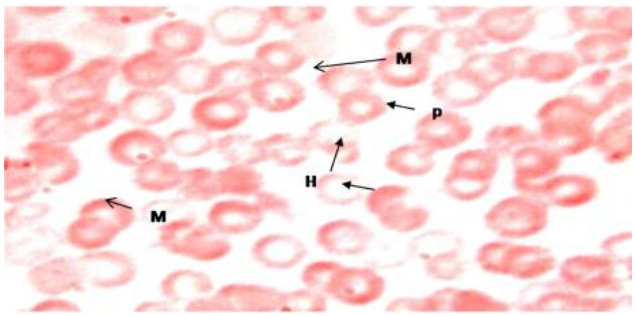

Figure 3: Blood smear of newborns of MW exposed pregnant females from 0.25 day of gestation showing overlapping erythrocytes, microcytes (M), poikilocytes (P) (Geimsa stain ; 100 X (oil immersion) x magnification).

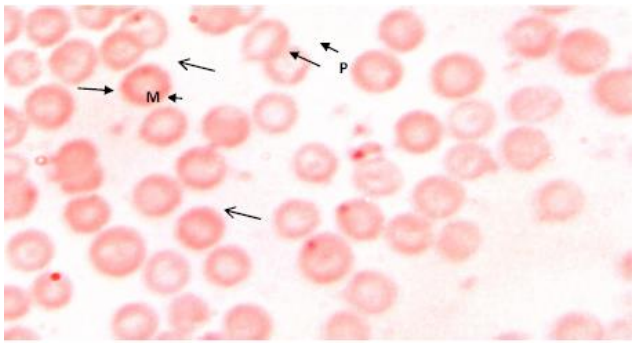

Figure 4: Blood smear of newborns of sham exposed pregnant females from 11.25 day of gestation showing round and discoid normocytes with no overlapping. (Geimsa stain; 100 X (oil immersion) x magnification).

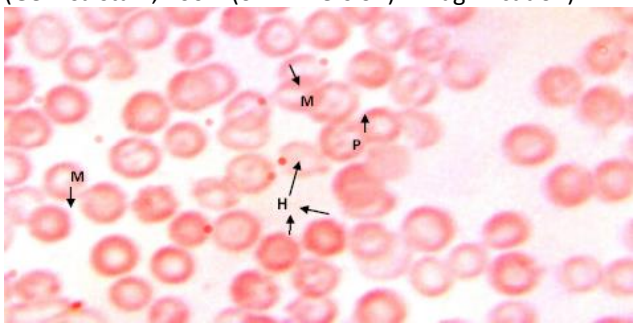

Figure 5: Blood smear of newborns of MW exposed pregnant females from11.25 day of gestation showing poikilocytes (P), microcytes (M), hypochromic (H) and

overlapping erythrocytes (Geimsa stain; 100 X (oil immersion) x magnification).

Blood smear of 3 week old mice whose mothers were exposed with 10 GHz from 0.25/11.25 day of gestation till term in the present investigation showed less severe damage in architecture of erythrocytes. Most of the erythrocytes retained their normal shape. Poikilocytosis and microcytosis were rare along with hypo chromic erythrocytes.

Table 3: Variations in the biochemical parameters of spleen of Swiss albino mice in follow-up at 3 week after 10 GHz MW exposure from 0.25 day and 11.25 day of gestation till term.

Treatments	Gestational day	LPO (nano mole /gm of protein)	GSH (nano mole /100gm)
Sham exposed	0.25	251.28±0.32	32.91±0.30
	11.25	251.76±0.067	32.38±0.22
MW Exposed	0.25	255.68±0.10*	30.01±0.45*
	11.25	254.89±0.98*	31.04±0.38*

Each value represents Mean ± SEM; Statistical comparison: Sham Vs MW exposed; Significance levels: *= P < 0.001; n=non-significant differences

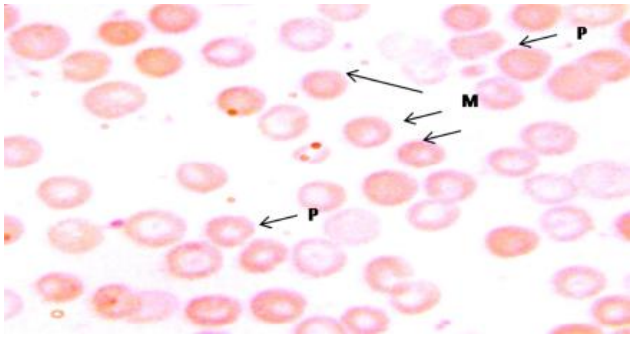

Figure 6: Blood smear of newborns of sham exposed pregnant females from 0.25 day of gestation showing round and discoid normocytes with no overlapping at age of 3 weeks (Geimsa stain; 100 X (oil immersion) x magnification).

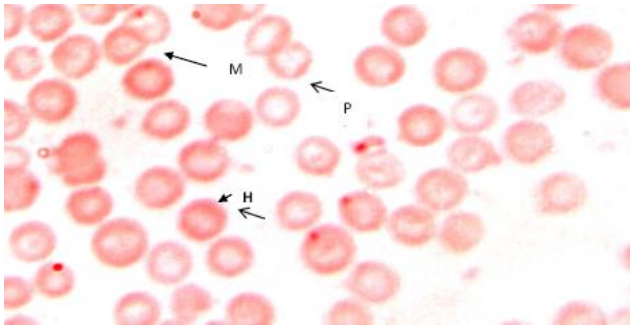

Figure 7: Blood smear of newborns of MW exposed pregnant females from 0.25 day of gestation showing no overlapping showing, poikilocytes (P), microcytes (M), hypochromic (H) at age of 3 weeks (Geimsa stain; 100 X (oil immersion) x magnification).

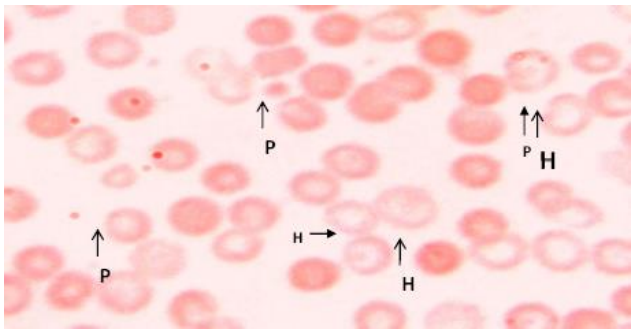

Figure 8: Blood smear of newborns of MW exposed pregnant females from 11.25 day of gestation showing hyporchromic erythrocytes (H), poikilocytes (P) at age of 3 weeks (Geimsa stain; 100 X (oil immersion) x magnification).

DISCUSSION

Nowadays, EMF and its hazardous or beneficial biological effects are subject to many studies on humans and animals. The environmental factors that are most critical to the optimal development of the embryo are those that occur during the prenatal period. Most of the environmental factors including radiations can affect the litter size of an animal. Post irradiation decrease in the litter size is a function of embryonic death. In the present investigation exposure of females to microwaves from 0.25/11.25 days of gestation produced no appreciable change in the litter size compared to sham. This clearly indicates that no prenatal death occurred. Studies have reported no influence in number of rat fetuses, resorptions, and sex ratios after gestational exposure to 1.95 GHz radiations.[12] Similarly; the effects of prenatal (*in utero*) exposure to Wi-Fi signals (2.45 GHz) on pregnancy outcomes have also reported that there was no difference in litter size in exposed mice compared to unexposed mice.[13] In the present study non-significant difference in crown rump length was observed in newborns of sham exposed and microwave exposed mice irrespective of gestation day exposure (0.25/11.25). This shows that 10 GHz exposure did not evoke any change in crown rump length of mice newborns. Non-significant alterations in fetal development after exposing pregnant females to different intensities (2.45 GHz and 100 MHz) have also been repported.[14,15] In the present study, fetuses prenatally exposed to microwave did not show any external morphological malformations. The results are in line with those who reported that there was no effect on the developing chick embryos exposed to 2450 MHz of microwave at power density 28mW/cm^2 for 100 minute daily from day 6 to day 15 of pregnancy.[16]

Growth retardation in newborn animals is a common and predictable consequence of irradiation *in utero*, the degree of retardation depends on the dose of radiation administered. The fetal bodyweight is a sensitive indicator for analyzing the growth retardation effects of intrauterine radiation. The whole gestational period is sensitive to radiations for weight loss of delivered newborns. Early gestation day exposure was more

harmful in this study as duration of exposure was more. Postnatal reduction in bodyweight directly expresses the radiation induced growth retardation of fetuses. Radiation induced cell death was probably responsible for decrease in bodyweight. The observed decrease in the mean body weight of the newborns prenatally exposed to microwave is probably due to impairment of blood flow to the placenta and reduced uterine blood flow by the effect of microwaves thus leading to reduced transport of nutrients and oxygen to the fetal circulation. This hypothesis agrees with the study which found a greater decrease in utero placental blood flow in pregnant rats following 915 MHz microwave exposure at 0.6mW/cm^2, which might result in abnormal pregnancy outcome, including abortion.[17] Decrease in the weight of newborns exposed to ELF-EMF compared to unexposed mice has also been reported.[18] Findings of the present study are also in line with those who exposed female rats during their gestation period (for three weeks) to Wi-Fi signals and concluded that there was no influence of Wi-Fi signal exposure on rat fetuses as evident by lack of malformations.[19] Similarly the effects of prenatal exposure to Wi-Fi signals (2.45 GHz) on pregnancy outcomes were studied and concluded that there was no difference in bodyweight in exposed mice compared to unexposed mice.[13] The effects of gestational exposure to 1.95 GHz radiations on rat fetuses were studied and it was conclude that 1.95 GHz have no influence on fetus body weight.[12] Along with negative effect of EMFs on development, various studies have reported effects of EMFs (50-60 Hz) on blood biochemical parameters in mammalian models.[20,21,22] The reduction of the weight of newborn mice may be justified with induction of apoptosis by 10 GHz microwave exposure by formation of free radical such as reactive oxygen species.

Blood and blood parameters are believed to be one of the primary particles that come in contact with radiofrequency /EMF. Blood being ions are likely to react with induced EMF generated by EMF charges. Measurements of blood parameters are most important means to determine the health status of experimental animals. [23] There are not many differences between the blood cells of neonate and adult but we found some distinguished characters which differentiate both morphologically and numerically in the blood cells from birth to adult. At birth the red blood cells are large, irregular showing different shapes and sizes in the peripheral blood smear of mice neonate as mice grows the shape and size of red blood cells becomes normal. As evident in the present investigation RBC's appear larger in size (Fig 2 & 4) in neonates born to Sham exposed from 0.25/11.25 gestation day compared to 3 week old mice (fig 6 & 8) whose RBCs attained almost original sizes at 3 week of age. In the present investigation MW exposure from different days of gestation (0.25/11.25 days) resulted in severe damage to erythrocytes (fig 5). The damage was more severe in neonates born to females exposed from 0.25 day of gestation compared to exposure from 11.25 day of gestation (fig 7). Exposure to 10 GHz microwaves altered the appearance and caused structural changes in the blood of one day old neonate mice and 3 week old mice.

CONCLUSION

10 GHz microwave exposure during pregnancy has a general cytotoxic effect on the total body weight blood of newborns and 3 weeks aged mice. It has no effect on litter size and crown rump length. 10 GHz microwave exposure may reduce the function of the hematopoetic system, as indicated by structural c hanges in blood cells. Therefore, using microwave producing instruments viz cell phones, Wi-Fi, laptops, microwave ovens etc should be limited during pregnancy. The early stage of gestation is important because the side effects of microwave radiations are more prominent.

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Source of Support: Nil, **Conflict of Interest:** None.