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Full Length Research Paper

Effects of thirty minute mobile phone irradiation on morphological and physiological parameters and gene expression in pregnant rats and their fetuses

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We investigated the potential effects of 30 min irradiation from a mobile telecommunication system. 60 pregnant rats divided to three groups; the first serve as the control (G1, n = 20), the second (G2, n = 20) and third (G3, n = 20) were exposed to electromagnetic fields (EMF) from 1st to 20th (G2) and from 7th to 16th (G3) day of gestation respectively. The implantation sites, corpora lutea, living, dead and reabsorbed fetuses were counted and recorded. Liver of pregnant rats and their fetuses were used to isolate a total RNA for quantification of Msx1 and Cx43 genes. Our result shows that abortion and partial abortion rate increased in G2 (30 and 25%) and G3 (10 and 20%) compared to 5 and 0% in G1. The body weights and fetal body length of fetuses were decreased in treated groups. Skeletal system abnormalities included short and curved tails absent of 13th rib and wavy ribs and absent of caudal vertebrae were observed in G2 and G3 compared to G1. The lowest relative expression of Ms x 1 and Cx43 (0.6 and 0.2) were found in from exposed mothers. Slight difference was found in the expression level between the exposed mothers (G2 and G3) compared to the control group (G1). This study suggests that exposure to EM fields during pregnancy could alter some morphological and physiological parameters and gene expression in pregnant rats and their fetuses.

Key words: Mobile signals, pregnancy, rats, fetus, physiological parameters, reproduction, skeletal system, gene expression.

INTRODUCTION

Mobile phones, also called cellular phones or handles, are now an integral part of modern life. The widespread use of mobile phones has been accompanied by the installation of an increasing number of base station antennas on masts and buildings. In recent years, increased public awareness and scientific research have questioned to what extent the non-thermal exposure to low intensity electromagnetic fields may affect the health, reproduction, well-being and behavior of humans and other organisms. Some researchers and national committees badvised more stringent safety standards, based on experimental data with reported biological effects from chronic non-thermal exposures (Hyland, 2000; Belyaev, 2005a, b).

Electromagnetic fields (EMF) are all around us. We need them to see, to listen to radio and watch television, to communicate using mobile phones, and we generate them every time we turn on a light switch or use an electric appliance. Specific absorption rate (SAR) is a measurement of how much electromagnetic radiation is absorbed by body tissue while using a mobile phone (http://www.mobile-phonesuk.org.uk/sar.htm).

The SAR value shows how much heat energy is absorbed in the head area from the mobile phone. The SAR value, specifically, is measured in the case of mobile phones because it is the best indicator of exposure of the user to radiation. When the SAR value is

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not more than two watts per kilogram, tissues are not heated significantly.

An electromagnetic field is a generic term for fields of force generated by electrical charges or magnetic fields. Under certain circumstances EMF can be considered as radiation when they radiate energy from the source of the fields. Electromagnetic waves periodically change between positive and negative. The speed of the changes, or the number of changes per second, is called the frequency and is expressed in hertz (1 Hz = 1 full cycle of change per second). Watts per kilogram (W/kg); unit of specific absorption rate (http://www.mobile-phonesuk.org.uk/sar.htm)

Although, some epidemiological studies, found that some types of abnormalities were seen more frequently in the children of women who had operated VDTs during their pregnancies compared to those who had not, there were no significant differences between the two groups (Brent et al., 1993; Schnorr et al., 1991; Trassner and Arnolds, 1992; Brandt and Nielsen, 1990; Chernoff et al., 1992; Tenforde and Kaune, 1987). Almost all of the studies reported significantly increased rates of growth retardation in animal fetuses exposed to EM fields.

A number of studies have been performed using mammals, and most of these have failed to find any consistent effects on fertility and reproductive performance, or on embryonic, fetal and postnatal development (Brent, 1992; NRC, 1996).

Up to now, several works by gene knockout mice have been issued to explain the basis of the pathogenesis of body defects related with some congenital human birth defects. In particular, mutations identified in some of the genes for transcription factors, cell signaling molecules, and proteases are associated with various degrees of developmental abnormalities (Dunker and Krieglstein, 2002; Brewer and Williams, 2004). Msx1 one of the genes encode the homeodomain transcription factors. Msx genes have been reported to play roles during various organo-genesis (Bei and Maas, 1998; Satokata et al., 2000; Zhang et al., 2003). Mice with homozygous mutations in both Msx1 and Msx2 die in late gestation with severe craniofacial malformations, including exencephaly, cleft palate, agenesis of teeth, and unossified calvarial bones (Bei and Maas, 1998; Satokata et al., 2000).

Loss of Msx1 expression resulted in mice with cleft secondary palate, agenesis of alveolar tissue of mandible and maxilla, abnormalities of the nasal, frontal, and parietal bones which all contain neural crest-derived tissues of the first branchial arch (Satokata and Maas 1994, Winograd et al., 1997). Msx1 plays a role in erythropoietic differentiation since low or null gene expression increased the level of progenitors and decreased the number of differentiated erythroid cells. Msx1 appears to act in mesenchymal cells since in Msx1-/- embryos the hematopoietic cells were abnormal and the cells that supported hematopoietic development in bone marrow were missing (Lazzarotto-Silva et al., 2005).

Cx43-deficient animals also exhibited retarded ossification of the clavicles, ribs, vertebrae, and limbs, and poor cell to cell diffusion of calcein resulting in skeletal abnormalities (Lecanda et al., 2000) where Cx43 is the major gap junction protein present in osteoblasts (Schirrmacher et al., 1992; Civitelli et al., 1993; Donahue et al., 1995). Gap junctions are involved in many phases of embryonic development and patterning, including heart morphogenesis, left–right asymmetry and limb patterning (Levin, 2001; Houghton, 2005). Moreover, without connexins, none of the bone cells function properly (Stains and Civitell 2005).

This study was designed to evaluate the teratogenic effect of EM fields emitted by mobile phones, on morphological and physiological parameters and gene expression in pregnant rats and their fetuses where gene expression is a unique way of characterizing how cells and organism adapt to changes in the external environment (Zhao et al., 2007).

MATERIALS AND METHODS

Sixty pregnant rats (Sprague-Dawley) divided into three groups (20 each), first serve as the control (G1), the second (G2) and third (G3) were exposed to EMF emitted from two mobile phones (GSM 1800) with absorption rate (SAR) 2.02 W/kg for 30 min/day from 1st to 20th (pregnancy period) and from 7th to 16th day (organogenesis time) of gestation respectively. The rats were kept inside an experimental chamber has size of 50 x 30 x 20 cm at room temperature between the mobile phones. The distance between mobile phone and animals is less than 10 cm. At the 20th day of gestation fetuses were removed from the uteri and examined. Liver of pregnant rats and their fetuses were used to isolate a total RNA for quantification of Msx1 and Cx43 genes by real time PCR.

The reproductive cycles of every rat which were kept in cages at room temperature, were followed for 15 days. Every female rat determined to be in estrus or proestrus phase of their cycles were mated with male rats in the same cage for one day and those which had sperm in their vaginal smears were considered to be at the day zero of their pregnancies. 20 of the 60 pregnant rats which were kept in a simple cage without any EM field exposure during the study were the control group (G1). The remaining pregnant rats divided into two groups and were exposed to EMF emitted by two pairs of mobiles each with 1.01 W/kg 30 min/day SAR. One group exposed for 30 min every day from 1st to 20th day of gestation (G2). The second group exposed for 30 min from 7th to 16th day of gestation (G3). All pregnant females were observed daily throughout gestation for mortality and general appearance. Maternal body weights were measured on gestational day 0, 6, 9, 12, 15, and 20.

At the 20th day of gestation Blood samples were collected from the eye, using orbital sinus technique (Sanford, 1954). Few drops of blood were collected in heparinized tubes for the hematological studies. The other portion of blood was collected and allowed to clot, then serum was separated by centrifugation at 3000 rpm for 20 min. and the clear non haemolysed serum was collected, divided into several aliquots and stored at -20°C until assayed.

Blood cell counts and hemoglobin (Hb) content were determined according to the methods adopted by Dacie and Lewis (1991). Red blood cell (RBC) and total leucocytic count (WBCs) were estimated using “Improved Neubauer hemocytometer”.

Table 1. Oligonucleotide primers used for real-time RT-PCR analysis.

Gene	Sequences Product	Size
GAPDH NM_017008	F: GGCTCTCTGCTCCTCCCTGTTCTA R: TGCCGTTGAACTTGCCGTGG	242
Msx1 NM_031059	F: GCCTGCACCCTACGCAAGCA R: AGCAGGCGGCAACATTGGCT	261
Cx43 NM_012567	F: TCCTTTGACTTCAGCCTCCAAGGAG R: GCAGACGTTTTCGCAGCCAGG	279

Serum Aspartate transaminase (AST) and Alanine (ALT) were determined colourimetrically according to the method of Reitman and Frankel (1957). Serum, urea and creatinine were determined according to the method of (Bartel, 1972). All of the pregnant rats were sacrificed by ether anesthesia on the 20th day of gestation and fetuses were removed from the uterus. The implantation sites, corpora lutea, living, dead and reabsorbed fetuses were counted and recorded. All living fetuses were weighed and evaluated for externally visible abnormalities. 50% of the fetuses were fixed in 96% ethanol and their soft tissues were removed in 1.0% KOH solution. After staining with Alcian blue- Alizarin Red-S combined technique, skeletal system was examined (Mcleod, 1980). Data obtained from the study and the control groups were compared statistically by ANOVA test.

RNA isolation and real-time reverse transcription polymerase chain reaction

Total RNA was extracted from liver of rat's mothers and its fetus using analytic jena bio solution (innuPREP RNA Mini Kit. Germany). For reverse transcription (RT), first strand complementary DNA (cDNA) was synthesized from RNA by using a cDNA Synthesis Kit (RevertAidTm First Strand cDNA Synthesis Kit, Fermentas,) according to the manufacturer's instructions.

After RT at 42°C for 60 min, polymerase chain reaction (PCR) was performed using a Jena Bioscience PCR-101 Taq Master Mix (Jena bioscience, Germany) according to the manufacturer's protocol. The specific primer pairs used in this study are listed (Table 1). Serially diluted cDNA samples were used as standards. After an initial denaturation step of 5 min at 95°C, 35 cycles of amplification for Msx1 and Cx43 primer pair, respectively, were carried out. Each cycle included a denaturation step, 30 sec at 95°C; an annealing step, 30 sec at 56°C; and an elongation step, 30 sec at 72°C. Final elongation temperature was 72°C for 5 min. Relative levels of gene expression were measured by QuantiTect SYBR Green PCR kit (Qiagen, Clinilab, Egypt) according to the manufacturer's instructions using Mx3000 instrument (Stratagene). The expression levels of Msx1 and Cx43 gene were normalized to the level of GAPDH gene expression in each sample.

RESULTS

Morphological parameters

There was no litter or fetus with major or minor abnormalities of any system in the control group. On the other hand effects were observed in EMF exposed groups as rate of abortion and partial abortion, fetal body weight, placenta weight and fetal death. External and skeletal examination of fetuses reported significant difference in incidence of fetal malformation between EMF exposed and control group. The rate of abortion and partial abortion increased in G2 compared to G3 (30 and 25% versus 10 and 20%) compared to 5 and 0% in G1 (Table 2).

The body weights of fetuses in G2 and G3 were statistically highly significantly decreased (3.02 and 2.13 g respectively) compared with that of G1 (3.54 g). Also the fetal body length was decreased than that of the control group (Table 2). There were significant differences in the body weights and weights of uteri for pregnant rats between groups (Tables 2 and 3, Figure 1). Fetuses had different abnormalities, hematoma, cleft palate, and paralyses in fore and hind limbs (Table 3).

Skeletal system abnormalities included short and curved tails, absent of 13th rib and wavy ribs and absent of caudal vertebrae. Variations of the skeletal system were ossification retardation of sternebrea, lumbar, sacral and caudal vertebrae, ossification retardation of ribs and ossification retardation or un-ossification of fore and hind limbs (Table 4, Figures 2 and 3).

Hematological studies

In the present study, however, hematological examination was added to the routine developmental toxicity study, since clinical pathology values can be used as markers for maternal toxicity. The results show clearly that exposure of pregnant rats to EMF during gestation period from 1st to 20th and from 7th to 16th day gestation did not produce any hematological significant effects in dams (Table 5). However, the estimation of hemoglobin content, red and white blood cell count reveals that EMF causes some hematological disorders in fetuses (Table 6). The average concentration of hemoglobin in G2 (10.6 g/dl) significantly decreased than that of G3 or G1 (11.6 and 11.5 g/dl) respectively. The RBCs and WBCs were significantly different than the control group. The WBCs in G2 was the highest, however, the RBCs of this group is the lowest one.

Liver and kidney function

Exposure to EMF from 1st to 20th day of gestation

Table 2. Effect of electromagnetic filed on the pregnant rats and their fetuses.

Group	Number of pregnant rats	Number of Complete abortion (%)	Partial abortion	Number of scarify (%)	Average weight of uteri± SE	Numberof implantation sit(/mother)	Number of living fetuses (%)	Number of reabsorbed fetuses (%)	Average body wt. of fetuses ±SE	Average body length of fetuses
G1	20	1(5%)	-	19(95)	38.62±3.69	146(7.68)	145(99.31)	1(0.67)	3.54±0.03	4.87(±0.02)
G2	20	6(30%)	5(25%)	16(70)	***28.49±2.63	94(5.87)	82(87.23)	12(12.77)	***2.13±0.23	***2.69(±0.1)
G3	20	2(10%)	420%	18(90)	**33.00±3.46	105(5.83)	96(91.43)	9(8.57)	**3.02±0.10	**3.86(±.05)

One way analysis of variance (ANOVA); **P Value <0.001 ; ***P Values <0.0001.

Table 3. Average body weights of pregnant rats and rate of malformation of fetuses.

Group	Pregnant rat			Effect on fetuses			
	Number of pregnant rats	Average increase in body weight at 20th day of gestation	Rate of increase in body weight (%)	Number of live fetuses (%)	Hematoma %)	Malformed limbs	
						Fore limb (%)	Hind limb (%)
G1	20	45.25±0.13	23.54	145 (99.31)	2 (1.38)	-	-
G2	20	29.8±0.14**	15.73	82 (87.23)	27 (32.93)	11 (13.41)	15 (18.29)
G3	20	35.26±0.23*	18.156	96 (91.43)	17 (17.71)	7 (7.29)	11 (11.96)

One way analysis of variance (ANOVA). Values are means ±SD; *P Values is <0.05 considered significant; ** P Values is <0.001 considered highly significant.

induced a significant increase ($p<0.05$) in serum creatinine (0.69 ± 0.01 mg/dl) compared to the control group (0.54 ± 0.01 mg/dl). But, exposure to EMF from 7th to 16th day of gestation caused no significant increase in serum creatinine of pregnant rats with mean value (0.55 ± 0.01 mg/dl) compared to the control ones (Table 7). On the other hand, the two groups exposed to EMF showed significant increase in serum urea level of pregnant rats with mean values of 5.30 ± 0.05 and 5.73 ± 0.06 mg/dl in G2 and G3 respectively compared with 4.005 ± 0.01 mg/dl of the control group.

Concerning liver function, EMF induced significant increase ($p<0.05$) in serum glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) level with mean values (5.98 ± 0.07 and 6.42 ± 0.06 mg/dl) and (5.57 ± 0.03 and 5.47 ± 0.02 mg/dl) in G2 and G3 respectively compared to 4.6 ± 0.05 and 4.03 ± 0.07 mg/dl for the control group.

Quantitative real-time PCR confirmation of selected transcripts

Two genes (Msx1 and Cx43) were quantified in independent samples from the three groups (G1, G2 and G3) by quantitative real-time PCR (Figures 4 and 5). The lowest relative expression of Msx1 (0.61) was found in fetus (G2 fetus) resulted from mothers exposed to EMF from 1st to 20th day of gestation (G2) followed by fetus (G3 fetus) resulted from mothers exposed to EMF from 7th to 16th day of gestation (G3). Slightly lower relative expression were found in exposed mothers (G2 and G3) compared to the control group mothers (G1). The expression profile of the Cx43 followed the same trend, as the lowest relative expression (0.2) was found in fetus (G2 fetus) resulted from mothers exposed to EMF from 1st to 20th day of gestation (G2) followed by fetus (G3 fetus) resulted from mothers exposed to EMF from 7th to 16th day of gestation (G3). Slight difference was found in the expression level

Figure 1. Uterus of control pregnant rats at 20th day of gestation (left) vs. uterus of pregnant rats exposed to EMF from 7th to 16th day of gestation.

Table 4. Effects of EMF on the skeletal system of fetuses.

Group		G1	G2	G3
No. of fetuses		70	70	70
No. of litters		19	16	18
Of litters has sk. Abnormal (%)		10.526	62.5	44.44
Ossification retardation	Skull (No.) (%)	5 (7.14)	25 (35.71)	42 (60)
	Stern bra (No.) (%)	2 (2.86)	27 (38.57)	47 (67.14)
	Ribs (No.) (%)	1 (1.43)	15 (21.43)	22 (31.43)
	Fore and hind limbs bones (No.) (%)	1 (1.43)	25 (1.43)	45 (64.28)
Wavy ribs (No.) (%)		00	5 (7.14)	2 (2.86)
Absent bones	13th ribs (No.) (%)	00	5 (7.14)	12 (17.14)
	Caudal vertebrae (No.) (%)	00	9 (12.86)	15 (21.43)
	5th stern bra (No.) (%)	00	7 (10)	11 (15.71)
	Metacarpals (No.) (%)	00	16 (22.86)	23 (32.86)
	Metatarsals (No.) (%)	00	19 (27.14)	25 (35.71)

between the exposed mothers (G2 and G3) compared to the control group (G1).

DISCUSSION

An electromagnetic field consists of an electrical part and a magnetic part. The electrical part is produced by a voltage gradient and is measured in volts/meter. The magnetic part is generated by any flow of current and is measured in tesla. Both types of field give biological effects, but the magnetic field is more damaging since it penetrates living tissue more easily. Magnetic fields as low as around one microtesla (a millionth of a tesla) can produce biological effects. For comparison, using a mobile (cell) phone or a personal digital assistant (PDA) exposes you to magnetic pulses that peak at several tens of microtesla (Jokela et al., 2004; Sage et al., 2007), which is well over the minimum needed to give harmful effects. Because mobile phones are held close to the body and are used frequently, these devices are potentially the most dangerous sources of electromagnetic radiation that the average person possesses (Goldsworthy, 2007).

A person's exposure to a mobile phone is measured in terms of SAR. This is a measure of the rate of energy deposition in a person's body during a call and is expressed in watts per kilogram (W/kg). SAR values for the most widely used phones range from 0.1 to 1.2 W/kg. Radio frequency (RF) fields penetrate tissues to depths

Figure 2. Skeletal system of the control fetuses (left) versus skeletal s ystem of fetus maternally exposed to EMF from 1st to 20th day of gestation showing absent of 13th rib and shortens of 12th &11th ribs.

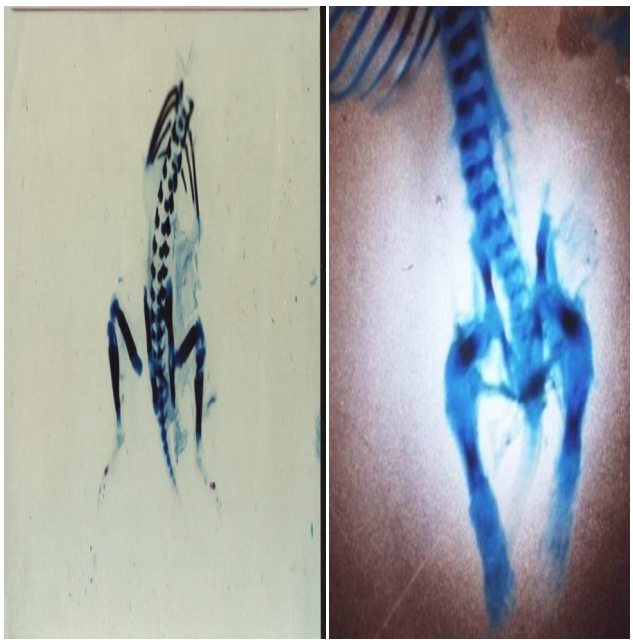

Figure 3. Hind limbs and vertebral column of control fetus at the 20th day of gestation (left) versus hind limbs and vertebral column of fetus maternally exposed to EMF from 1st to 20th day of gestation, showing sever lack of ossification.

that depend on the frequency. At mobile phone frequencies the RF energy is absorbed to a depth in tissue of about one centimeter. RF energy absorbed by the body is converted into heat that is carried away by the body. All established adverse health effects are caused by heating. While RF energy can interact with tissues at

Table 5. Effects of EMF exposure on hematological values of pregnant rats.

Groups	Control	From 1st to 20th day of gestation	From 7th to 16th day of gestation
No. of animales	20	20	20
WBC (×10/ml)	7.59±2.53	9.3±0.24	8.02±0.93
RBC (×10/ml)	5.74±0.36	5.09±0.18	5.73±0.11
HGB (g/dl)	13.4±0.57	12.6±0.60	12.6±0.86

Values are means ±SD; WBC, white blood cells; RBC, red blood cells; HGB, hemoglobin; In significant difference at P>0.05 level when compared with the control group.

Table 6. Effects of EMF exposure on hematological values of fetuses at the 20th day of gestation.

Group	Shame control	From 7th to 16th day of gestation	From 1st to 20th day of gestation
No. of animals	30	30	30
WBC (×10/ml)	9.68±2.66	10.75±0.85*	11.04±0.22*
RBC (×10/ml)	5.86±0.26	4.93±0.10*	4.09±0.16*
HGB (g/dl)	11.5±0.48	11.6±0.68	10.6±0.59*

Values are means ±SD; WBC, white blood cells; RBC, red blood cells; HGB, hemoglobin; * indicates significant difference at P<0.05 level when compared with the control group

Table 7. Effects of EMF on liver and kidney function of pregnant rats.

Groups	GOT	GPT	Urea	Creatinine
G1 (Control)	4.60 ± 0.05	4.03 ± 0.07	4.00 ± 0.01	0.54 ± 0.01
G2	5.98* ± 0.07	5.57* ± 0.03	5.30* ± 0.05	0.69 ± 0.01
G3	6.42* ± 0.06	5.47* ± 0.02	5.73* ± 0.06	0.51 ± 0.01

*P Values of <0.05 is considered significant.

levels that do not cause significant heating, there is no consistent evidence of adverse health effects at exposures below the international guideline limits (http://en.wikipedia.org/wiki/Mobile phone radiation and health).

Most of the epidemiological studies which assessed the effects of EMF on human fetuses reported significantly increased risks of spontaneous abortion and growth retardation (Schnorr et al., 1991; Brandt and Nielsen 1990; Chernoff et al., 1992; Juutilainen, 2005). Some of those studies stated that renal, genital, cardiac and skeletal systems and chromosomal abnormalities had been observed more frequently in the fetuses of the Visual Display Terminal (VDT) operators than in those of the normal population (Brent et al., 1993; Trassner and Arnolds, 1992; Chernoff et al., 1992). However, neither the increased risks of these abnormalities were observed to be statistically significant, nor was any correlation found between a special type of abnormality and exposure to EMF. Calcium ions bound to the surfaces of cell membranes are important in maintaining their stability. They help hold together the phospholipids molecules that are an essential part of their make-up (Ha, 2001). Without these ions, cell membranes are weakened and are more likely to tear under the stresses and strains imposed by the moving cell contents (these membranes are only two molecules thick). Although, the resulting holes are normally self-healing they still increase leakage while they are open and this can explain the bulk of the known biological effects of weak electromagnetic fields (Goldsworthy, 2007).

Leaks in the membranes surrounding lysosomes can release digestive enzymes, including DNAase (Goldsworthy, 2007). This explains the serious damage done to the DNA in cells by mobile phone signals. Panagopoulos et al. (2007) showed that exposing adult Drosophila melanogaster to a mobile phone signal for just six minutes a day for six days broke into fragments the DNA in the cells that give rise to their eggs and half of the eggs died. Diem et al. (2005) also found significant DNA fragmentation after exposing cultured rat and human cells for 16 hours to a simulated mobile phone signal (http://tinyurl.com/yxy4ld). It shows that exposing human cells nfor 24 h to simulated mobile phone signals gave

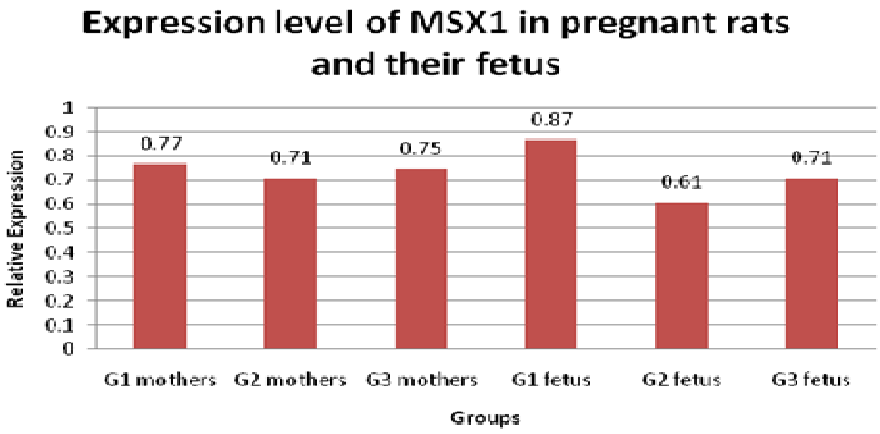

Figure 4. Expression level of MSX1 gene in control group (G1) and treated groups (G2 and G3).

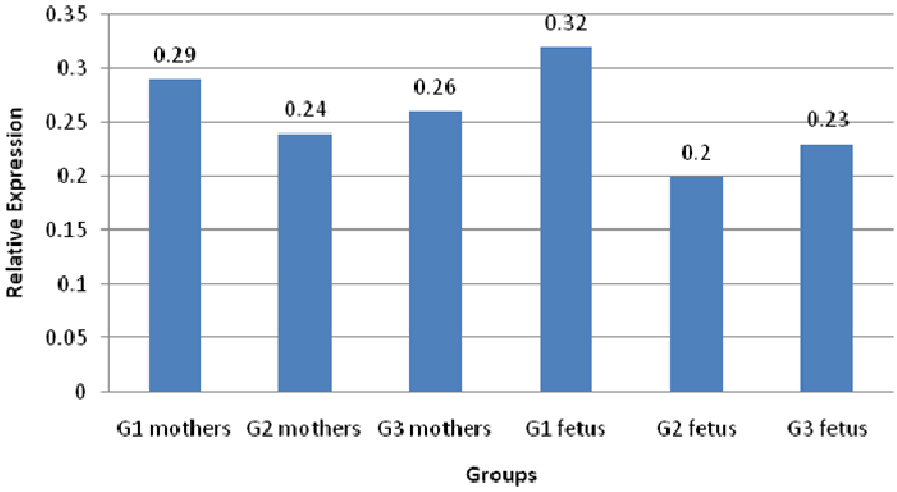

Figure 5. Expression level of CX43 gene in control group (G1) and treated groups (G2 and G3).

DNA fragmentation similar to that due to the gamma rays from a radioactive isotope! (Gamma rays also make lysosome membranes leak).

Increased risks of growth retardation and skeletal system abnormalities were observed in most of the studies which investigated the effects of EMF on animal fetuses (Juutialainen and Saali, 1986; Zusman et al., 1990; Martin, 1988). Other study conducted by Stuchly et al. (1988) found that exposure to EMF with an intensity of more than 100 mG significantly increased the risks of variations and minor abnormalities, without affecting the risk of major abnormalities, while Huuskonen et al. (1993) observed an increased risk of variations, minor and major abnormalities of the skeletal system of rat fetuses. Another study, performed by Juutialainen et al. (1986) which used chicken embryos, reported that exposure to EMF with a frequency of 16 kHz or more significantly increased the risk of growth retardation and abnormalities of the skeletal system, however, those with lower frequencies did not have any significant effect. A comprehensive study, which also used chicken embryos, found that not only the risk of abnormalities of skeletal system and growth retardation were increased significantly by EMF, but also correlated positively with the increased intensities and frequencies of the EMF (Juutilainen et al., 1986). In contrast with these, Cameron et al. (1985) showed that exposure to EMF resulted in growth retardation but did not cause any structural abnormalities of the rat fetuses, while in another study which used the same animal, neither any abnormality nor any variation in the skeletal system was observed to be increased significantly (Cameron et al., 1985; Rommereim et al., 1987).

In this study, fetuses 44.44% of the 18 litters and

62.5% of 16 litters exposed to EMF in G2 and G3 respectively were found to have skeletal system abnormalities (Table 4). These abnormalities include Ossification retardation (skull, stern bra, Ribs and Fore and hind limbs) Wavy ribs and Absence bones (13th ribs, Caudal vertebrae, 5th stern bra, Metacarpals and Metatarsals).

When the effects of EMF on bone metabolism were assessed it was found that EMF increased the entry of calcium ions into the cells of bone tissue and altered the expansion of ossification (Adey, 1981). Because of these effects, EMF has been used in the treatment of bone fractures of adult humans (Andrew et al., 1984). In contrast, some studies in the literature reported that exposure to EMF had resulted in retardation of ossification of the bone tissue (Chernoffet al., 1992; Tenforde and Kaune, 1987; Huuskonen et al., 1993). In the study of Hasan et al. (1998) not only the light microscopic examinations showed that ossification and segmentation of bone tissues were significantly retarded but also electron microscopic examinations revealed that maturation and differentiation of cartilage tissues were significantly delayed in fetuses exposed to EMF. These findings suggested that EMF could also affect the differentiation of soft tissues.

Another major effect of electromagnetic radiation is the leakage of free calcium ions, either through the cells' external membranes or those surrounding internal 'calcium stores'. This can have dramatic effects on many aspects of metabolism and explains most of the mysterious but well-documented physiological effects of electromagnetic fields. These include stimulations of growth, an increased risk of cancer, symptoms suffered by electro-sensitive humans and why using a mobile phone while driving makes you four times more likely to have an accident (Goldsworthy, 2007).

Matthews (1986) reported that exposing nerve and muscle cells to calcium concentration about 10 to 20% below normal made them significantly more excitable, which fits with our hypothesis. These findings also explain many of the symptoms of hypocalcaemia. Hypocalcaemia is a medical condition, usually caused by a hormone imbalance, in which the concentration of ionized calcium in the blood is abnormally low. By removing bound calcium from cell membranes, it should (and does) give similar effects to electromagnetism.

In the present study the White Blood cells (WBCs) in-significant increased in pregnant rats of two experimental groups than that of the control group. However, WBCs of the fetuses maternally exposed to EMF from 1st to 20th day and 7th to 16th day of gestation were significant increased than that of the control group. Also there were non-significant decrease in Red blood Cells (RBCs) and hemoglobin (Hb) concentration of the pregnant rats but in their fetuses there were significant decrease.

In the developmental toxicity study of EMF in rats, Stuchly et al. (1988) observed a decrease in maternal lymphocyte count in the 66 mT (660 mG) exposure group, but it was within the normal range. Ryan et al. (1996) found a reduction in body weight in both sexes of fetuses in the 0.2 mT (2 G) exposure group.

It has been stated that weak electromagnetic fields can remove calcium from cell membranes and make them leak. If we theorize about the mechanism, we can explain many of the seemingly weird characteristics of bio-electromagnetic responses. These include why weak fields can be more effective than strong ones, why low frequencies are more potent, why pulses do more damage than sine waves and what is special about 16Hz. The following hypothesis was proposed by Goldsworthy (2006).

These data Demonstrating the importance of Cx43 for limb development, as confirmed before using antisense oligonucleotides inhibition of Cx43 expression in the chick embryo which resulted in limb malformations, including truncation of the limb bud, fragmentation into two or more domains, or complete splitting of the limb bud into two or three branches (Becker et al., 1999; Green et al., 1994). These data implicate that Cx43 plays a very important role in osteogenic function and stated that, the genetic deletion of Cx43 results skeletal ossification abnormalities. Therefore, the lack of Cx43 causes a generalized osteoblast dysfunction and this in accordance with what reported by (Lecanda et al., 2000). Other role was found also for Msx1 as an important gene for embryo development as reported by (Tesfaye et al., 2010). In addition Msx genes may modulate the regulation of type I collagen possibly affecting the formation of extracellular matrix (ECM) development (Dodig et al., 1996; Alappat et al., 2003). Moreover, mice with homozygous mutations in both Msx1 and Msx2 die in late gestation with severe craniofacial malformations, including exencephaly, cleft palate, agenesis of teeth, and unossified calvarial bones (Bei and Maas, 1998; Satokata et al., 2000). Our results concerning expression level of both Cx43 and Msx1 are in accordance with what was stated before about the importance of these genes in organogenesis and embryo development. Our study is the first to clarify the effect of EMF on the expression level of some important genes for embryo development.

Conclusion

Data obtained in this study suggested that exposure to EM fields during pregnancy could alter the normal development and increase the risk of variations in the skeletal system of rat fetuses. In addition, expression level of Msx1 and Cx43 was affected by the treatment. Although, it was believed that further studies are needed to confirm this result, more epidemiological studies should also be planned to evaluate the effects of EM fields created by mobile phone on human fetuses.

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