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Mobile Phone RF-EMW Exposure to Human Spermatozoa: An *in vitro* Study

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Abstract.- Present study was conducted to determine the effect of 1 hour mobile phone radio frequency electromegnatic waves (MP-RF-EMW) exposure, with and without vitamin E supplementation, on sperm malondialdehyde (MDA) concentration, motility percentage and gradation. Healthy fertile subjects (n=22) of 20-35 years of age were recruited from Faisalabad and surroundings. The mean fast progressive (A) sperm percentage and non-motile percentage (D) were significantly (P<0.01) lower (4.28%) and higher (4.06%), respectively, in MP-RF-EMW exposed group compared to un-exposed group. Mean MDA (µmol/ $10x10^6$ sperms) concentration from mobile EMW exposed group was non significantly (P>0.05) the highest compared to both the other groups, non exposed and treated exposed. In MP-RF-EMW exposed group, spermatozoal MDA concentration was the significantly (r=-0.5816; p=0.0057) negatively correlated with sperm total motility percentage. It is concluded that mobile phone radiation likely to induce the sperm membranous lipid peroxidation through ROS mediated cascade of reactions, consequently, decreases the fast progressive motile sperms percentage, and increases the non-motile sperms percentages. Whereas vitamin E has shielding effects against MP-RF-EMW generated ROS subsequently diminished the MDA concentration and improves the motility gradations. Hence it is mandatory to avoid the sperms from EMW exposure during sperm preparation for IVF and use of vitamin E supplemented mediums.

Key words: Malondialdehyde, Sperm motility, motility gradation, mobile phone radiation.

INTRODUCTION

Several studies have shown the effect of radio-frequency-electromegnatic waves (RF-EMW) on different biological systems (Aitken *et al*., 2005; Balci *et al*., 2007; Capri *et al*., 2004; Carlo and Jenrow, 2000; Friedman *et al.,* 2007; Ozgur *et al.,* 2010), however not a single study explained the mechanism of action of these radiations very effectively (Feychting *et al*., 2005). These effects ranged from the molecular level such as up-regulation of apoptosis genes expression, (Zhaoa *et al*., 2007), change in enzyme activity, (Nylund and Leszczynski, 2004), to the tissue level, for instance, increased risk of brain tumor (Hardell *et al*., 2002), disruption of long-term memory (Wang and Lai, 2000), and an increase in chick embryo mortality (Ingole and Ghosh, 2006). However, the role of cell phone exposure on sperm motility (Fejes *et al*., 2005), morphology (Wdowiak *et al*., 2007), viability, chromatin integrity and instability has lately addressed, and proposed a reduction in male fertilizing potential (Agarwal *et al.,* 2009; Aitken *et al*., 2005; Diem *et al*., 2005). These detrimental effects on biological systems were considered as adverse thermal effects of mobile phone generated energy (Black and Havnick, 2003) in terms of specific absorption rate (SAR). In United States, the SAR of cell phones varies from 0.12–1.6 W/kg. After animal experimentations it was concluded that SAR threshold level (4W/kg) render by RF, likely to induce the adverse thermal effects such as on unit core body temperature increment (1°C) (Barnes and Greenebaum, 2007). On the other hand studies suggested that RF-EMW emitted from cell phones have no adverse thermal effects (Anderson and Rowley, 2007; Straume *et al*., 2005; Yan *et al*., 2007). In most parts of world including Pakistan, the cellular phones work at 900-1800 MHz frequencies. Various *in vitro* studies using animal models have consistently demonstrated oxidative stress in different tissues (kidney, endometrium, eye, testis, brain, myocardial tissues and so on) in response to cell phone radiation (Meral *et al*., 2007; Oktem *et al*., 2005).

Reactive oxygen species (ROS) are produced continuously by spermatozoa, and neutralized by antioxidants present in the semen (Sharma *et al.,* 1999; Irmak *et al.,* 2002). However, when ROS production exceeds the capacity of antioxidants, a

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state of oxidative stress is created. A recent study of Agarwal *et al.* (2009) demonstrated a decrease in ROS-TAC score by cell phone exposure was concomitant to increased oxidative stress. Human spermatozoa plasma membranes are considered highly sensitive to ROS-induced damage, and an important pathophysiological mechanism in human male infertility (Agarwal *et al*., 2003). Briefly, ROS triggered the lipid peroxidation and caused the loss of membrane integrity, increased cell permeability, enzyme inactivation (Nylund and Leszczynski, 2004), structural damage to DNA (Agarwal *et al.,* 2008a), and cell death (Cummins *et al*., 1994; Halliwell *et al.* (1994). Malondialdehyde (MDA), an end-product of lipid peroxidation, represents the level of lipid peroxidation. High lipid peroxidation as represented by MDA levels likely is to cause the changes in sperm and diminish fertility (Geva *et al*., 1996).

Current study was designed to observe the effect of 1 hour cell phone radiation on human sperm motility pattern and its relationship with spermatozoa MDA concentration. Furthermore it was also aimed at observing the effect of *in vitro* supplementation of vitamin E prior to EMW exposure on sperm motility profile and MDA concentration.

MATERIALS AND METHODS

Semen samples

Semen samples were collected from 22 volunteer male individuals of age group 20-35 years by masturbation after an abstinence period of 48 hours and were liquefied completely within 15-30 minutes, at room temperature.

Each semen sample was divided into nine aliquots of 200 µl each, three replicates for each of the two experimental and control group, one of the experimental groups was supplemented with 50µl vitamin-E (α-tocopherol) solution (1mM) in Ringer-tyrode as described by Verma and Kanwar (1999).

Control or non-exposed group	aliquot A1, A2 &A3
MP-RF-EMW exposed group	aliquot B1, B2 &B3
Vitamin E supplemented MP-RF-EMW exposed group	aliquot C1, C2 &C3

Exposure of semen aliquots to cell phone radiation

All aliquots were placed for 10 minutes on clean table without any intervention of other electric instruments. Aliquots A replicates were isolated and remaining aliquots were exposed (10cm distance as used by Erogul *et al*., 2006) to radiation emitted from a mobile phone in talk mode (Nokia 1112; Ufone GSM; 900 MHz frequency) for 1 hour.

Sperm motility gradation

Aliquots A3, B3 and C3 were used in the assessment of sperm motility gradation as criterion described by Erogul *et al.* (2006). For this purpose semen sample was thoroughly mixed with sterile plastic pipette (3ml) and a drop was positioned on a clean glass slide (25.4x76.2mm) and placed cover slip immediately. Extra fluid was removed with blotting paper. Motile sperms were counted in three different sites of slides under microscope at 400X objective. Among 100 sperms four types of sperm were separately counted according to their movement pattern; A-fast progressive, B-slow progressive, C-non-progressive and D-non-motile.

Biochemical analysis

Separation of seminal plasma

Seminal plasma of semen aliquots 1 and 2 of A, B and C was separated by centrifugation at 1800xg for ten minutes. The sperm pellets were re-suspended in 1 ml phosphate buffered saline (PBS) (pH 7.47). The sperm concentration in suspensions was adjusted at 10×10^6 spermatozoa per 1 ml with Horwell chamber.

Measurement of lipid peroxidation

Lipid peroxidation was measured by determining the malondialdihyde (MDA) production.

Each sperm suspension (1ml) was mixed with 2ml of TBA reagent (15% trichloroacetic acid + 0.25N HCl) in sterile caped tube and incubated in water bath at 100C^o for 15 minutes. After cooling, the suspension was centrifuged at 1000xg for 10 minutes and optical density of supernatant was measured 0 at 535nm by spectrophotometer (Model U-2800). The MDA concentration (µmol/10×10^6 sperms) was determined by the specific absorbance coefficient (1.34×10^5 mole/ml).

Statistical analysis

Sperm motility grades and spermatozoan MDA concentrations from different experimental groups were statistically compared by Student's t-test and Kruskal Wallis test was applied to compare the medians (GraphPad Prism V).

RESULTS

Sperm motility gradation

In present study the all subjects (n=22) fulfill the WHO motility criteria >40% motile sperms were included. Sperm motility grading was performed according the WHO criteria, fast progressive (A), slow progressive (B), non-progressive (C) and non-motile (D) (Table I). Among all experimental groups, non MP-EMW exposed, MP-EMW exposed and vitamin E supplemented MP-EMW exposed group, the highest motile sperms frequency percentage were observed in fast progressive motility grade (A). The fast progressive motility grade sperm frequency was significantly decrease in exposed group when compared with non-exposed (4.28%, t=4.002, P=0.0009) and supplemented-exposed group (3.53%, t=1.385, P=0.042). Alternatively, non-motile (D) sperm frequency was increased significantly in exposed group when compared with both non-exposed (4.06%, t=3.961, P=0.001) and supplemented-exposed group (4.40%, t=1.441, P=0.003). A negligible increase (1.26 and 1.02% respectively) in slow progressive and decrease (0.39 and 0.30% respectively) in non-progressive sperms frequency was observed in exposed group than non-exposed and supplemented-exposed groups. There was no considerable difference in all motility grades from non-exposed and supplemented-exposed groups.

Lipid peroxidation

In present study the oxidative stress derivate MDA was also estimated in the spermatozoal pellet of all treated groups and control group of semen sample from all subjects. Mean MDA (µmol/ $10x10^6$ sperms) concentration of semen samples from mobile EMW exposed group was non significantly (P>0.05) higher compared to both the other groups (Fig. 1).

Table I.- Mean fast-, slow-, non-progressive and non-motile sperms percentage in non-EMW exposed, EMW exposed and vitamin E supplemented EMW-exposed groups' *in vitro* conditions

Motility grades	Non-EMW-exposed group	EMW-exposed group	Vit. E supplemented EMW-exposed group
Fast progressive (A)	24.772± 1.613	20.494± 1.735[a***]	24.022± 1.560[b**]
Slow progressive (B)	14.250± 1.439	12.994± 1.002	14.017± 1.282
Non-progressive (C)	12.339± 0.898	12.725± 1.258	12.425± 0.927
Non-motile (D)	48.452± 2.542	52.515± 2.782[a***]	48.111± 2.539[b**]

Values are Mean±SEM percentage of sperms.
[a]non-EMW-exposed group vs. EMW exposed group, Vit. E supplemented EMW exposed group.
[b]EMW-exposed vs Vit. E supplemented EMW exposed group
P<0.05*, P<0.01**, P<0.001***.

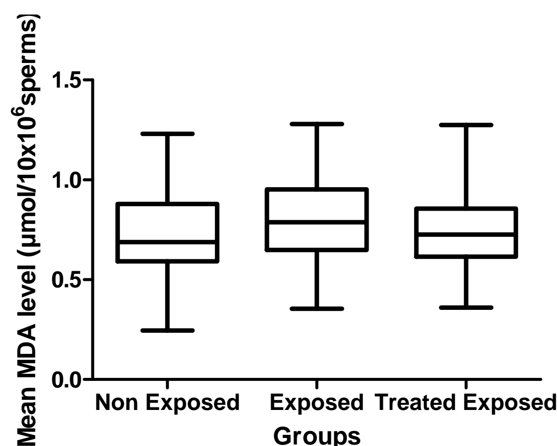

Fig. 1. Percentage of mean MDA level in EMW non exposed, exposed and vitamin E treated exposed spermatozoa determined by using the spectrophotometer. Values are medians (—) with interquartile (25–75%) and minimum and maximum values ( ). The non parametric H test of Kruskal and Wallis confirmed that the medians were not significantly varied in all groups.

MDA concentrations of individual semen aliquots from all experimental and control group were negatively correlated with total motility of the sperms (Fig. 2). In non EMW exposed group and EMW exposed group the MDA concentration had significant negative correlation with the total

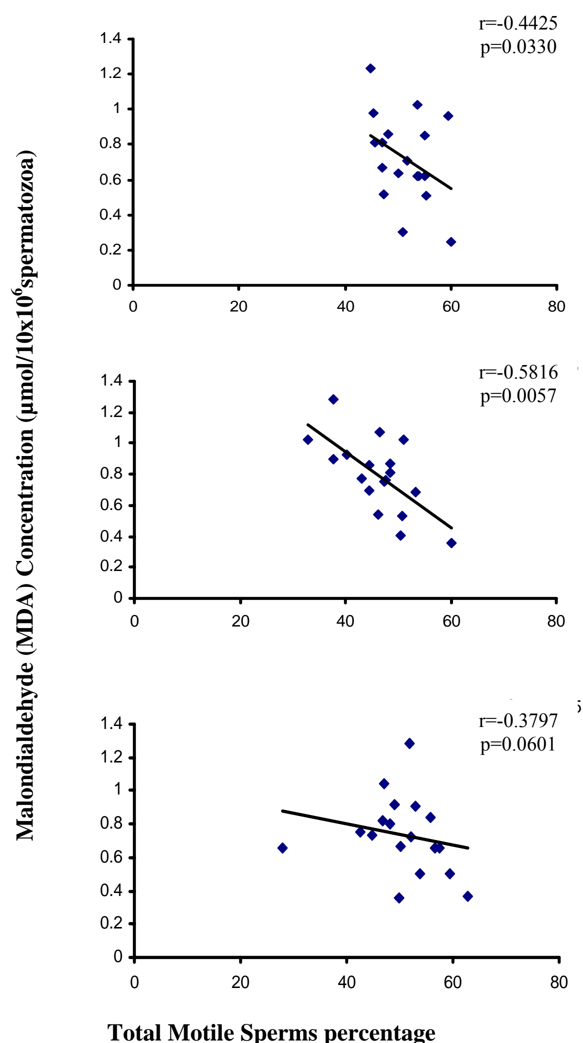

Fig. 2. Correlations between total sperm motility and MDA concentration in Non-exposed group (Top) presented the negative linear trend, EMW-Exposed group (Middle) and Vitamin E-supplemented-exposed group (Bottom).

motility of the sperms (r=-0.4425, P=0.0330; r=-0.5816, P=0.0057, respectively), however, in vitamin E supplemented EMW exposed group it was non significantly (r=-0.3797, P=0.0601) negatively correlated with the total number of motile sperms. The highest MDA concentration was seen in the sperms with low motility from mobile EMW exposed group and was least in sperms with high total motility from the non EMW exposed group, however difference between un-exposed and vitamin E supplemented EMW exposed group was not appreciable. Medians did not vary in non-exposed, exposed and treated exposed groups (H=1.478, P=0.4775).

DISCUSSION

Various studies have recently illustrated the possible influences of cell phone radiation on human spermatozoa. Present study was conducted to give the possible mechanism through which cell phone radiation diminish sperm motility. A section of this experiment was also performed to study the protective effect of vitamin E supplementation against lipid peroxidation induced by cell phone radiation.

Previously various studies have claimed that the prolong usage of cell phones may have negative effects on sperm parameters *i.e.,* motility, viability and morphology (Agarwal *et al.,* 2008a,b; Davoudi *et al*., 2002; Erogul *et al.,* 2006; Falzone *et al.,* 2008; Fejes *et al.*, 2005; Makker *et al.,* 2009; Wdowiak *et al.,* 2007). Wdowiak *et al.* (2007) reported 65.7% men, not using cell phones, had >50% (WHO category A + B) sperm motility, whereas frequently cell phones user were only 35.4% men with same sperm motility.

An important finding of current *in vitro* study was a decrease in total sperm motility in EMW-exposed (1 hour exposure) aliquots (46.18±1.33%) as compared to non-EMW-exposed aliquots (51.37±1.34%). Recently, an *in vitro* pilot study has also shown the reduction of mean percentage of total motile sperms in cell phone exposed (1 h) group from 52.11±18.34% to 48.62±17.36% (Agarwal *et al.,* 2009). Another study of cell phone exposure duration concluded that sperm motility declined with increase in exposure time, 67.80±6.16, 64.57±8.47, 54.72±10.97 and 44.81±16.30, % on 0, <2, 2-4 and >4hr./day, respectively (Agarwal *et al.,* 2008a).

In current study comparison between exposed and non-exposed groups showed statistically significant (p<0.05) changes in sperm motility in fast progressive and non-motile grades of sperm motility. Motility of fast progressive sperms was

diminished (non-exposed: 24.78±1.61% vs exposed: 20.49±1.73%), while mean number of non-motile sperms per hundred total sperms increased in EMW-exposed group (non-exposed: 48.48±2.54% vs exposed: 52.52±2.78%). Similar results have been reported by Erogul *et al.* (2006), in their *in vitro* study, EMW exposure from cellular phone for 5 minutes significantly decreased the percentage of fast progressive sperms (from 13.6±10.2% to 9.1±7.9%) and slow progressive sperms (from 43.7±19.4% to 33.9±20.6%). Whereas non-motile sperm percentage was increased in exposed (50.6±22.7%) group compared to non-exposed (35.9±2.6%) group.

In another study 371 men were presenting for an infertility workup, duration of possession and daily transmission time of cell phones correlated negatively with the proportion of rapid progressive motile spermatozoa ($r = -0.12$ and $r = -0.19$, $P<0.01$) and positively with the proportion of slow progressive motile spermatozoa ($r = 0.12$ and $r = 0.28$, $P < 0.01$) (Fejes *et al.,* 2005).

Recently, however a significant decrease in straight-line velocity and beat-cross frequency of sperms was also observed at 5.7 W/kg SAR (specific absorbance rate) generated by 900 MHz pulsed radiation. No significant change in any kinetic parameters, including MMP and progressive sperm motility found at 2.0 W/kg (Falzone *et al.,* 2008). Another study revealed that Isocitrate dehydrogenase may change its activity by the mobile phone radiation and leads to decrease production of ATP in mammalian cells (Nylund and Leszczynski, 2004). This diminished production of ATP also has its additive affect to decreased sperm motility gradation under EMW exposure by mobile phone.

The most remarkable finding of the present study was an increase in spermatozoal MDA concentration in EMW-exposed semen aliquots, from 0.72±0.06 to 0.79±0.06 μmol/10x10^6 sperms. In contrast previously MDA concentration was estimated very low 0.09±0.04 nmol/10x10^6 sperms (Tavilani *et al.,* 2005) and 0.40±0.06nmol/ 10x10^6 sperms (Zarghami and Khosrowbeygi, 2005) in asthenospermic samples.

It was also speculated that radiofrequency electromagnetic waves emitted from cellular phones may lead to increase in ROS production. Whenever ROS production exceeds from the capacity of antioxidants, a state of oxidative stress created (Agarwal *et al.,* 2008b). It has been observed that 1800 MHz mobile phone radiation at SAR of 4 W/kg for 24 hours increased intracellular ROS, by NADH oxidase stimulation (Friedman *et al.,* 2007), and damage DNA significantly.

It is well known fact that ROS play major role in lipid peroxidation of spermatozoal membranes (Aitken *et al*., 1989; Griveau *et al.,* 1995; Sharma and Agarwal, 1996; Tavilani *et al.,* 2006; Twigg *et al.,* 1998; Williams and Ford, 2005; Zarghami and Khosrowbeygi, 2005) since their plasma membranes are enriched with polyunsaturated fatty acids, particularly docosahexaenoic acid with six double bonds (Khosrowbeygi and Zarghami, 2007; Tavilani *et al.,* 2007). It has been illustrated that reactive oxygen species produced under the influence of cell phone radiation caused an increase in lipid peroxidation.

Therefore in present study, increase in spermatozoal MDA level by cell phone radiation may be considered parallel to ROS production oxidative stress. MDA is only one of the several degradation products generated during the lipid peroxidation process of sperm membranes (Gomez *et al.,* 1998) and had inverse correlation with sperm motility (Hsieh *et al.,* 2006). It is previously reported that asthenospermic patients had negatively correlated high concentration of MDA than normospermic subjects (Suleiman *et al.,* 1996; Tavilani *et al*., 2005; Zarghami and Khosrowbeygi, 2005).

In current study, sperm MDA concentration inversely correlated with the percentage of total sperm motility in EMW-exposed group and suggests that the spermatozoa which became immotile by the influence of cell phone radiation underwent lipid peroxidation. Motility of spermatozoa depends on the integrity of the mitochondrial sheath, of which the phospholipids are major component. If fatty acids in these phospholipids are oxidized by free radicals, mitochondrial membrane potential will be abolished and consequently, motility will be impaired (Falzone *et al.,* 2008; Fraczek *et al.,* 2001; Nabil *et al.,* 2008).

Vitamin E has capability to act as powerful

antioxidant and is very necessary to save body cells from free radicals. The present study also evidenced one more perspective in the field of reproductive medicine that vitamin E significantly improved sperm motility in *in vitro* condition and caused an appreciable decrease in the spermatozoal MDA concentration in vitamin E supplemented-EMW-exposed semen aliquots. It has been previously suggested that oxidative stress in semen is a consequence of ROS inflation compared to antioxidants (Agarwal *et al.,* 2009) that protect ejaculated spermatozoa from oxidative stress (Fraczek *et al*., 2004). Exposure of spermatozoa to ROS has been associated with cellular injury, which includes DNA damage and lipid peroxidation (Potts *et al*., 2000).

In conclusion, current study provided an evidence that mobile phone radiation is likely to enhance the lipid peroxidation of sperm membranous polyunsaturated fatty acids through enhanced ROS production, by the NADH oxidase stimulation and diminished ATP production, subsequently increasing the MDA concentration, which decreases the fast progressive motile sperm percentage, and increases the non-motile sperms percentages. It was also clearly observed that after vitamin E supplementation, low level of MDA was estimated even though exposed to the EMW of mobile phone for the same time as in first experiment. Hence increase of vitamin E intake in our daily life may decrease the risk of sperm damage caused by cell phone radiation.

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