ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 2, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

RESOLVING THE ENIGMA OF EFFECT OF MOBILE PHONE USAGE ON SPERMATOGENESIS IN HUMANS IN SOUTH INDIAN POPULATION

SUTTUR S MALINI*

Department of Studies in Zoology, Molecular Reproductive & Human Genetics Laboratory, University of Mysore, Manasagangotri, Mysore, Karnataka, India. Email: drssmalini@gmail.com

Received: 26 September 2016, Revised and Accepted: 18 October 2016

ABSTRACT

Objective: This study was aimed at to evaluate the possible risk of radiofrequency and electromagnetic waves of mobile phones on spermatogenic impairment and functional capacity of the spermatozoa along with oxidative stress, DNA damages, and hormone profile among mobile phone users.

Methods: Mobile phone users were classified into three groups are 1-5, 6-10, and above 10 hrs/day, respectively, based on the exposure to electromagnetic radiation. Blood and semen samples are collected with informed consent letter. The semen samples used to carry out to the physical examination such as volume, liquefaction time, color, odor, pH, and viscosity, and functional status of the spermatozoa was carried out such as nuclear chromatin decondensation test, hypo-osmotic swelling test, and acrosomal intactness test. Seminal plasma was used for to evaluate the oxidative stress markers superoxide dismutase (SOD) activity, reactive oxygen species (ROS) levels, and total antioxidant capacity (TAC). Blood serum was used to estimate the level of follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone. DNA collected from blood used for DNA ladder assay.

Results: In the present investigation, both physical and microscopic examinations were negatively correlated with mobile phone usage. No variation exists in functional status of spermatozoa. Oxidative stress markers such as the presence of ROS, enzymatic scavengers such as SOD and TAC showed no statistical variations between control group and mobile phone users and even no variations in hormone profile such as testosterone, FSH, and LH of users of mobile phone compared to normal reference values.

Conclusion: In conclusion, though the literature has suggested that mobile phone use alters semen parameters, functional status of spermatozoa, increased oxidative stress, with subsequent sperm DNA damage in humans. The present study deviates from previous study stating nil impact of mobile phones on spermatogenetic impairment in humans.

Keywords: Association of mobile phone usage, Spermatogenesis, ROS, Male infertility.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i2.15392

INTRODUCTION

Globalization and modernization made humankind to excessively depend on electronic gadgets on a day to day basis. It is impossible to imagine a present mankind without the usage of mobile devices and/or computers with Wi-Fi function. Since two decades, there has been a significant increase in cell phone usage throughout the world including developing countries. Cell phones operate using frequencies that differ by manufacturer and country, and concerns are growing about the possible hazardous effect of radiofrequency (RF) and electromagnetic waves (EMW) emitted by these communication tools on human health [1]. Negative impact of usage of the mobile phone on human health was reported by many researchers [2-8]. Male infertility is upcoming problem of the modern world rising in alarming rate globally [9]. In general, the quality of sperm in recent years has worsened throughout the world [10-12]. One of the biggest fears is that this RF-EMW may disturb testicular function and alter conventional and/or nonconventional sperm parameters [1]. A number of reports have suggested a possible link between cell phone use and decreased semen quality [13-16]. It is well known that infertile men are distinguished by abnormal semen characteristics. The majority of infertile or subfertile men are subjects with diminished sperm motility and DNA damage [17]. Emission of electromagnetic radiation from mobile phones, results in oxidative stress and DNA fragmentation can apparently lead to the development of different pathologies in spermatogenesis [18,19]. Leydig cells, seminiferous tubules, and spermatozoa are the main targets of the damage caused by mobile phones on the male reproductive tract. In particular, cell phone exposure reduces testosterone biosynthesis, impairs spermatogenesis, and damages sperm DNA [1]. Scrotal hyperthermia and oxidative stress are the main mechanisms by which the damage is generated [20]. Many animal studies have shown that EMW negatively interferes with the male reproductive system. However, similar studies are limited in humans, and the results obtained in the experimental animal may be replicated on humans with caution.

In this view, we made an attempt to evaluate the possible risk on spermatogenic impairment and functional capacity of the spermatozoa along with oxidative stress, DNA damages, and hormone profile among mobile phone users.

METHODS

The present investigation was carried on 50 male individuals of age between 25 and 50 years both married and unmarried irrespective of caste and religion from in and around Mysore, India. Informed written consent letters were taken from the all the participants. Study individuals were interviewed orally to collect information about their family, medical, reproductive histories which include the duration of active married life, sexual history, premature ejaculation, and psychological status of the subjects and lifestyle factors, dietary pattern. Questions associated to cell phone usage, duration of usage, daily standby position, and daily transmission times were recorded before semen analysis were carried out. Mobile phone users were classified

into three groups based on the exposure to electromagnetic radiation. Exposure hours are 1-5, 6-10, and above 10 hrs/day. The positions of mobile phones were in their trousers close to the testis. Individuals with chronic health issues, infertility, urinary infections, metabolic disorders, under medication, with supplementation of antioxidants, users of laptops were excluded from the study. Healthy male individual of age between 25 and 50 years without the usage of mobile phone was used as control subjects.

Semen sample collection and preservation

The semen samples were collected after 3-5 days of ejaculatory abstinence according to the WHO criteria [21]. Physical examination such as volume, liquefaction time, color, odor, pH, and viscosity were recorded. Microscopic examinations were carried out to study the count, vitality, motility, and morphology of the sperm according to the WHO guidelines [21].

Functional test for spermatozoa

Nuclear chromatin decondensation test (NCD) was carried out to check the ability of decondensation of nuclear chromatin *in vitro* in spermatozoa. Integrity of plasma membrane was performed using hypo-osmotic swelling (HOS) test. Quality of the acrosomal enzymes was analyzed using acrosomal intactness test (AIT). NCD and AIT were carried out by the modified method of Gopalkrishna [22]. HOS test as described by Jeyendran *et al*. [23]. Around 200 spermatozoa were scanned under microscope using a ×40 objective for each test.

Evaluation of oxidative stress markers superoxide dismutase (SOD) activity, reactive oxygen species (ROS) levels, and total antioxidant capacity (TAC) of seminal plasma

The principle for SOD activity estimation involves scavenging of superoxide radicals that are produced by photoreduction of riboflavin. These superoxide radicals are then allowed to react with hydroxylamine hydrochloride to produce nitrite. The nitrite, in turn, reacts with sulfanilic acid to produce a diazonium compound, which subsequently reacts with naphthylamine to produce a red azo compound whose absorbance was measured at 543 nm using multimode reader. The generation of ROS in the male reproductive tract has become a real concern in recent years because of their potential toxic effects at high levels on sperm quality and function [24]. ROS is highly reactive oxidizing agents. About 25-40% of infertile men have high levels of ROS in their semen [24].

Chemiluminescence's assay for ROS measurement

Liquefied semen was centrifuged at 300 ×g for 7 minutes, and the seminal plasma was separated. The pellet was washed with phosphate-buffered saline (PBS) and resuspended in the same washing media at a concentration of 20×10^6 sperm/ml. 400 µl aliquots of the resulting suspensions were used to assess basal ROS levels. 10 µl of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), prepared as 5 mM stock in dimethyl sulfoxide was added to the mixture and serve as a probe. A negative control was prepared by adding 10 µl of 5 mM luminol to 400 µl of PBS. Luminol is an extremely sensitive oxidizable substrate that has the capacity to react with a variety of ROS at neutral pH. The reaction of luminol with ROS results in production of a light signal that is read in luminometer as arbitrary light units.

TAC

TAC levels of the subjects were estimated by phosphomolybdenum method to infer the total amount of antioxidants present in the subjects. Samples were mixed with 5% trichloroacetic acid as a reducing agent and incubated at room temperature for 10 minutes. To the supernatant 1 ml TAC reagent ($0.6H_2SO_4$, 28 Mm sodium phosphate and 4 µm ammonium molybdate) was added. The mixture was incubated in water bath at 95°C for 90 minutes. After cooling absorbance was read at 695 nm against blank and expressed as µg/ml. Standard curve was prepared using vitamin C and the amount of TAC present in the seminal plasma was estimated from the standard curve. The results were expressed as µg/ml.

DNA ladder assay

About 2 ml intravenous blood was collected by a trained medical practitioner from the infertile and control individual, and the same was transferred to an ethylenediaminetetraacetic acid (EDTA) Vacutainer. Extraction of genomic DNA was carried out from blood samples of the infertile individuals employing QIAamp DNA blood mini kit (Qiagen, Netherlands). The amount of DNA was quantified by spectrophotometry and further diluted to 100 ng concentration in Tris-EDTA buffer. Separated DNA was subjected for electrophoresis on a 2% agarose gel containing 1 µg/mL ethidium bromide and visualize by ultraviolet transillumination.

Estimation of hormones

The intravenous blood was collected and allowed to clot; serum was separated by centrifuging at 8000 rpm for 5 minutes at room temperature. The serum obtained was used to estimate the level of follicle stimulating hormone (FSH), luteinizing hormone (LH) (Erba, Germany), and testosterone (DRG, Germany). The readings were taken under ELX 800 Biomed micro-titer plate reader.

Statistical analysis

In the present study, SPSS (version 20) statistical software program was employed for data analysis. Results were provided as mean±standard deviation with standard error for continuous variables. Independent-sample t-test was performed to find a significant difference between groups. $p<0.05$ considered as significant difference.

RESULTS

In the present pilot investigation, Table 1 showed the analysis of semen parameters in mobile phone user. Both physical and microscopic examinations were negatively correlated with mobile phone usage. No variation exists in semen parameters of mobile phone users.

The analysis of functional competence of spermatozoa in mobile phone users was studied by employing sperm function tests which reveal the functional status of sperm with reference to acrosomal enzymes, sperm NCD stability, and membrane intactness such as NCD, HOS, and AIT. Statistically, values from mobile phone users for sperm function tests were not deviated from references values of the normal range (Table 2).

Oxidative stress markers such as presence of ROS, enzymatic scavengers such as SOD and TAC showed no statistical variations between control group and mobile phone users in (Table 3).

Table 4 showed even no variations in hormone profiles such as testosterone, FSH, and LH of users of mobile phone compared to normal reference values. In the present study, no DNA damages found in gel profile.

DISCUSSION

In recent years, there is drastic reduction of semen parameters such as the sperm count, motility, and morphology have been noted worldwide [10-12]. Researchers are undisputed about the negative impact of certain environmental factors such as smoking and alcohol abuse, exposure to xenobiotics, sedentary lifestyle, intake of high-calorie food, and increased local testicular temperature on spermatogenesis [10-12,25]. Consequently, the WHO reference values for fertility fitness of healthy individuals was redefined [21]. A number of studies have attempted to elucidate the effects of cell phone radiation on human sperm function using a direct approach that consists of exposure of direct or processed spermatozoa to RF-EMW for a variable length of time [1]. Many researchers reported the negative impact of mobile phone usage and impairment in semen parameters for instance sperm count, motility, viability, and normal morphology most likely due to oxidative damages and DNA damages [13-16,25,26]. However, reports from Yildirim *et al*. [27] were contradicting from previous work. Therefore, no certain conclusions can be drawn about the impact of mobile phone usage on fertility fitness. In contrast to the previous

Table 1: Analysis of semen parameters in mobile phone user

Parameters	Duration of mobile phone usage and contact with the body (hrs)	n	Mean	Standard error	F-value	p value
Volume	1-5	20	2.2	0.35	0.355	0.703^{NS}
	6-10	22	2.0	0.23		
	Above 10	5	1.7	0.11		
pH	1-5	20	7.9	0.09	0.372	0.691^{NS}
	6-10	22	8.0	0.06		
	Above 10	5	7.9	0.14		
Count	1-5	20	60.7	12.2	0.974	0.386^{NS}
	6-10	22	43.0	5.73		
	Above 10	5	50.0	8.73		
Motility	1-5	20	49.5	2.63	0.023	0.977^{NS}
	6-10	22	49.0	3.38		
	Above 10	5	48.0	5.83		
Morphology	1-5	20	12.5	1.65	0.480	0.622^{NS}
	6-10	22	11.3	1.68		
	Above 10	5	15.2	5.33		
Vitality	1-5	20	43.8	4.69	1.069	0.352^{NS}
	6-10	22	52.2	3.90		
	Above 10	5	43.4	10.44		

n: Number of individuals, NS: Nonsignificant

Table 2: Analysis of functional competence of spermatozoa in mobile phone user

Parameters	Duration of mobile phone usage and contact with the body (hrs)	n	Mean	Standard error	F value	p value
NCD	1-5	19	64.4	3.69	1.005	0.375^{NS}
	6-10	21	70.8	2.71		
	Above 10	5	66.0	6.78		
HOS	1-5	19	60.2	3.27	0.200	0.819^{NS}
	6-10	21	62.9	2.88		
	Above 10	5	60.2	8.24		
AIT	1-5	19	50.9	2.97	1.616	0.212^{NS}
	6-10	21	58.3	3.18		
	Above 10	5	50.0	7.35		

n: Number of individuals, NCD: Nuclear chromatin decondensation, HOS: Hypo-osmatic Swelling, AIT: Acrosome intactness test, NS: Nonsignificant

Table 3: Analysis of oxidative stress markers in semen samples of mobile phone user

Parameters	Duration of mobile phone usage and contact with the body (hrs)	n	Mean	Standard error	F value	p value
TAC	1-5	20	71.1	2.95	0.329	0.721^{NS}
	6-10	22	74.3	2.71		
	Above 10	5	73.0	5.10		
ROS	1-5	20	667.7	150.18	0.397	0.675^{NS}
	6-10	22	649.6	170.46		
	Above 10	5	388.1	122.50		
SOD	1-5	20	0.65	0.15	2.314	0.111^{NS}
	6-10	22	0.36	0.05		
	Above 10	5	0.28	0.09		

n: Number of individuals, TAC: Total antioxidant capacity, ROS: Reactive oxygen species, SOD: Superoxide dismutase, NS: Nonsignificant

Table 4: Analysis of hormone profile among mobile phone user

Parameters	Duration of mobile phone usage and contact with the body (hrs)	n	Mean	Std. Error	F value	p value
Testosterone	1-5	17	4.4	0.40	0.328	0.723*NS*
	6-10	20	4.27	0.27		
	Above 10	4	3.82	0.93		
FSH	1-5	17	5.06	0.63	4.302	0.021*NS*
	6-10	20	4.16	0.54		
	Above 10	4	8.40	1.83		
LH	1-5	17	3.77	0.46	1.826	0.175*NS*
	6-10	20	5.37	0.79		
	Above 10	4	6.10	1.42		

n: Number of individuals, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, NS: Nonsignificant

study, those who have supported adverse effect of mobile phone on semen parameters, our results were contradicting without any statistical significance for semen parameters and functional potency of spermatozoa (Table 1). Gorpinchenko *et al*. [28] demonstrate a significant decrease in sperm progressive motility and a significantly higher proportion of sperm with DNA damages could be due to decreased levels of catalase and glutathione peroxidase activity. In the present study, a nonsignificant association exists between cell phone users WHO reference values for semen parameters namely count, motility, vitality, volume, morphology, etc. Even functional competence of spermatozoa did not show any significant deviations in all three groups.

The seminal plasma antioxidant system is the sum of enzymatic stress markers such as SOD, catalase, and glutathione peroxidase and nonenzymatic stress markers, namely, ascorbate, urate, vitamin E, pyruvate, glutathione, taurine, and hypotaurine. Seminal oxidative stress develops as a result of imbalance between the generation of ROS and its scavenging activities. Spermatozoa are particularly susceptible to oxidative stress-induced damage because their plasma membrane contains large quantities of polyunsaturated fatty acids and their cytoplasm contains low concentrations of scavenging enzymes. Oxidative stress attacks not only the fluidity of the sperm membrane but also the integrity of DNA in the sperm nucleus. These markers have an effective system that can provide spermatozoa with a protective environment. In the present study, no variations exist in ROS, SOD, and TAC activity among mobile phone users (within the groups or as compared to the control) that could be one of the possible reasons why we were unable to record the negative impact of mobile phone usage.

The endocrine control of spermatogenesis is well-established factor, wherein testosterone, FSH, and LH have a significant role. LH and FSH are secreted by anterior pituitary basophile cell and called gonadotropins because they stimulate the gonads in male - the testes and in female - the ovaries. LH and FSH are essential for reproduction. Physiological effects of these two hormones are known only in the ovaries and testes, together they regulate many aspects of gondal function in both males and females [29]. In both sexes, FSH stimulates gamete (sperm or egg) production, whereas LH promotes the production of gonadal hormones [30], whereas testosterone is shown to modulate the most component of the male hormone. Hormone assay carried in this study is to make sure the discern effect of hormone in spermatogenetic impairment. However, fortunately hormone profile showed no significant variation with reference values indicating nil roles of electromagnetic radiations in disruption of reproductive hormones. So far, available literature about adverse effect of mobile phone was confined to western population. The studies were scanty and confined to *in vitro* condition in the Indian context. However, India is a country known for its uniqueness wherein exposure to xenobiotics are sporadic; the lifestyle is quite dynamic which also includes Yoga, Pranayama, and other maneuver along with meditation which scaffold mental stability. Indian dietary pattern enriched with ample of antioxidants which would have maintained stability in *in vivo* against ROS. Overall data which hold good for the western population not directly extrapolate for our study. Hence, in our study, there is no consensus on mobile phone RF-EMR radiation effects on human fertility. Additional well-designed investigations are needed to evaluate the real consequences of long-term employment of these devices.

CONCLUSION

In conclusion, though the literature has suggested that mobile phone use alters semen parameters, functional status of spermatozoa, increased oxidative stress, with subsequent sperm DNA damage in humans. The present study deviates from previous study stating nil impact of mobile phones on spermatogenetic impairment in humans. Nevertheless, a structured detailed study is necessary in large cohort to provide stronger evidence on cell phone usage and its association with sperm and testicular dysfunction since the existing literature has several technical limitations.

ACKNOWLEDGMENT

The authors are thankful to ICMR, New Delhi, for financial assistance. We are grateful to The Chairman, DOS in Zoology, Physicians and Volunteers; Authors are also thankful to lab members, MRHGL, University of Mysore. The authors declared that this study was supported by funds from ICMR, New Delhi, India.

REFERENCES

1. La Vignera S, Condorelli RA, Vicari E, D'Agata R, Calogero AE. Effects of the exposure to mobile phones on male reproduction: A review of the literature. J Androl 2012;33(3):350-6.
2. Salford LG, Brun A, Sturesson K, Eberhardt JL, Persson BR. Permeability of the blood-brain barrier induced by 915 MHz electromagnetic radiation, continuous wave and modulated at 8, 16, 50, and 200 Hz. Microsc Res Tech 1994;27(6):535-42.
3. Repacholi MH, Basten A, Gebski V, Noonan D, Finnie J, Harris AW. Lymphomas in E mu-Pim1 transgenic mice exposed to pulsed 900 MHZ electromagnetic fields. Radiat Res 1997;147(5):631-40.
4. Phillips JL, Ivaschuk O, Ishida-Jones T, Jones RA, Campbell-Beachler M, Haggren W. DNA damage in molt-4 T-lymphoblastoid cells exposed to cellular telephone radiofrequency fields *in vitro*. Bioelectrochem Bioenerg 1998;45:103-10.
5. Preece AW. Mobile phones and human heads. Neuro Report 2000;11(2):
6. Hardell L, Carlberg M, Hansson Mild K. Pooled analysis of two case-control studies on the use of cellular and cordless telephones and the risk of benign brain tumours diagnosed during 1997-2003. Int J Oncol 2006;28(2):509-18.
7. Pesonen M, Hämäläinen H, Krause CM. Brain oscillatory 4-30 Hz responses during a visual n-back memory task with varying memory load. Brain Res 2007;1138:171-7.
8. Hillert L, Akerstedt T, Lowden A, Wiholm C, Kuster N, Ebert S, *et al.* The effects of 884 MHz GSM wireless communication signals on headache and other symptoms: An experimental provocation study. Bioelectromagnetics 2008;29:185-96.
9. Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, Dohle G, *et al.* European Association of Urology guidelines on Male Infertility: The 2012 update. Eur Urol 2012;62(2):324-32.
10. Jørgensen N, Vierula M, Jacobsen R, Pukkala E, Perheentupa A, Virtanen HE, *et al.* Recent adverse trends in semen quality and testis cancer incidence among Finnish men. Int J Androl 2011;34(4 Pt 2):e37-48.
11. Geoffroy-Siraudin C, Loundou AD, Romain F, Achard V, Courbière B, Perrard MH, *et al.* Decline of semen quality among 10 932 males consulting for couple infertility over a 20-year period in Marseille, France. Asian J Androl 2012;14(4):584-90.
12. Burton A. Study suggests long-term decline in French sperm quality. Environ Health Perspect 2013;121(2):a46.
13. Davoudi M, Brossner C, Kuber W. The influence of electromagnetic waves on sperm motility. J Urol Urogynakol 2002;19:18-22.
14. Fejes I, Závaczki Z, Szöllosi J, Koloszár S, Daru J, Kovács L, *et al.* Is there a relationship between cell phone use and semen quality? Arch Androl 2005;51(5):385-93.
15. Erogul O, Oztas E, Yildirim I, Kir T, Aydur E, Komesli G, *et al.* Effects of electromagnetic radiation from a cellular phone on human sperm motility: An *in vitro* study. Arch Med Res 2006;37(7):840-3.
16. Agarwal A, Deepinder F, Sharma RK, Ranga G, Li J. Effect of cell phone usage on semen analysis in men attending infertility clinic: An observational study. Fertil Steril 2008;89(1):124-8.
17. Schulte RT, Ohl DA, Sigman M, Smith GD. Sperm DNA damage in male infertility: Etiologies, assays, and outcomes. J Assist Reprod Genet 2010;27(1):3-12.
18. De Iuliis GN, Newey RJ, King BV, Aitken RJ. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa *in vitro*. PLoS One 2009;4(7):e6446.
19. Hardell L, Carlberg M, Hansson Mild K. Epidemiological evidence for an association between use of wireless phones and tumor diseases. Pathophysiology 2009;16(2-3):113-22.
20. Deepinder F, Makker K, Agarwal A. Cell phones and male infertility: Dissecting the relationship. Reprod Biomed Online 2007;15(3):266-70.
21. World Health Organization. Laboratory Manual for the Examination

of Human Semen and Sperm-Cervical Mucus Interaction. New York: Cambridge University Press. p. 2010.
22. Gopalkrishnan K. Standardized procedures in human semen analysis. Curr Sci 1995;68:353-62.
23. Jeyendran RS, Van der Ven HH, Perez-Pelaez M, Crabo BG, Zaneveld LJ. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J Reprod Fertil 1984;70(1):219-28.
24. Garg V, Garg SP. Role of nitric oxide in male infertility. J Indian Acad Forensic Med 2011;33(1):65-8.
25. Erogul O, Oztas E, Yildirim I, Kir T, Aydur E, Komesli G, *et al.* Effects of electromagnetic radiation from a cellular phone on human sperm motility: An *in vitro* study. Arch Med Res 2006;37(7):840-3.
26. Boulos V, Hassan H. Do cell phones affect semen quality? Eur Urol Suppl 2013;12:843-4.
27. Yildirim ME, Kaynar M, Badem H, Cavis M, Karatas OF, Cimentepe E. The effect of radiofrequency-electromagnetic radiation on semen parameters. Eur Urol Suppl 2013;12:1113-5.
28. Gorpinchenko I, Nikitin O, Banyra O, Shulyak A. The influence of direct mobile phone radiation on sperm quality. Cent European J Urol 2014;67(1):65-71.
29. Hussein Z, Al-Qaisi J. Effect of diabetes mellitus Type 2 on pituitary gland hormones (FSH, LH) in men and women in Iraq. J Al-Nahrain Univ 2012;15(3):57-79.
30. Elaine N, Marieb RN. The endocrine system. In: Anatomy Physiology. Vol. 15. San Francisco: Benjamin Cummings; 2001. p. 512-8.