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The semen quality of the mobile phone users

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ABSTRACT. ***Background:*** **The increased use of mobile phones, the media's attention for general health, and the increase of idiopathic male infertility suggest to investigate the possible consequences of an excessive use of mobile phones on semen quality.** ***Aim:*** **To evaluate the conventional and some of the main biofunctional sperm parameters in healthy men according to the different use of the mobile phone.** ***Subjects and methods:*** **All the enrolled subjects in this study were divided into four groups according to their active cell phone use: group A= no use (no.=10 subjects); group B= <2 h/day (no.=16); group C= 2-4 h/day (no.=17); and group D= >4 h/day (no.=20). Among the subjects of the group D (>4 h/day), a further evaluation was made between the "trousers users"(no.=12) and "shirt users"(no.=8), and they underwent semen collection to evaluate conventional and biofunctional sperm parameters (density, total count, morphology, progressive motility, apoptosis, mithocondrial membrane potential, chromatin compaction, DNA fragmentation).** ***Results:*** **None of the conventional sperm parameters examined were significantly altered. However, the group D and the trousers users showed a higher percentage of sperm DNA fragmentation compared to other groups.** ***Conclusion:*** **These results suggest that the sperm DNA fragmentation could represent the only parameter significantly altered in the subjects who use the mobile phone for more than 4 h/day and in particular for those who use the device in the pocket of the trousers.**
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INTRODUCTION

There is a growth of interest about the problem of the mobile phone's use and its potential impact on the male fertility (1, 2). The increased use of mobile phones (3), the increased attention of the media for the general health (2), and in particular, in the case of the andrological diseases, the increase in the rate of idiopathic male infertility (without apparent causes) (4) represent in our opinion a justified reason to investigate this problem from a clinical point of view.

The data search on Medline using the following keywords, "mobile phone and male infertility" or "mobile phone and sperm" or "mobile phone and semen parameters", found a limited number of articles although well distributed among several studies concerning experimental models (5-12) and a series of clinical evidences (13-19). Recently, our group published a review on this topic (20), confirming the need to improve the knowledge through the clinical trials.

On the basis of these premises, the aim of this study was to evaluate the quality of the conventional and some of the main biofunctional sperm parameters of a selected series of healthy men comparing the results between the mobile phone "regular users" and "non-users".

Key-words: Mobile phone, semen quality, users.

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SUBJECTS AND METHODS

Design of the study

Observational study.

Subjects recruitment

Clinical evaluation of all patients consecutively referred to our center of Andrology in the year 2012.

Subjects selection

Sixty-three healthy and fertile men (all studied subjects induced pregnancy in the last year), with normal weight [body mass index (BMI) range 19.0-24.5 kg/m^2], aged between 18 and 35 yr, and non-smoking were carefully selected for enrollment in this study. The examined patients were selected from an original population (consisting of 250 men, aged between 18 and 35 yr and with BMI range 19.0-32.0 kg/m^2) observed with the opportunity of the andrological screening (regular prevention of male infertility) made in the past 2 yr in the institutes involved in the study. To exclude subjects with the concomitant presence of an andrological disease known as able to alter sperm conventional and biofunctional sperm parameters, a complete medical history was collected from each of them. All men with a negative anamnesis underwent a careful physical examination and laboratory (routine blood testing, sperm analysis, sperm culture, and urethral swabs) and ultrasound instrumental (scrotal and transrectal scans) evaluation. Men with systemic (21) and endocrine diseases (22), male accessory gland infection (23), past or present cryptorchidism (24) or varicocele (25), microrchidism (26), cigarette smoking (27), alcohol (28) and/or drug abuse, and recent hormonal treatment were excluded.

In particular, the threshold values used to exclude cases of hypogonadism and hypothyroidism were the following: total testosterone <3 ng ml–1 or 10.4 nmol l–1 (29); thyrotropin (TSH) >4.5 μU ml–1 (30).

Overweight or obese subjects and smokers were excluded from

the study for the possible consequences that these conditions may have on conventional and unconventional sperm parameters, as demonstrated in other studies of our and other groups (31-33).

Table 1 shows the clinical, hormonal and ultrasound characteristics of the sample and the four subgroups.

All subjects enrolled in this study underwent semen collection to evaluate conventional and biofunctional sperm parameters.

The patients were divided into four groups according to their active cell phone use (assessed through a specific questionnaire): group A= no use (no.=10 subjects); group B= <2 h/day (no.=16 subjects); group C= 2-4 h/day (no.=17 subjects); and group D= >4 h/day (no.=20 subjects). Among the subjects of the group D (>4 h/day) a further evaluation was made between the "trousers users"(no.=12 subjects) and "shirt users"(no.=8 subjects) ("trousers users" were considered the men who usually carry the phone in the pocket of the pants and "shirt users" were considered the men who usually carry the phone in the pocket of the shirt). All the examined subjects have used the same mobile phone device [UMTS/HSDPA/HSUPA (850, 900, 1900, 2100 MHz)] during the last year and reported that the device was turned on during the 24 h of the day (data obtained by questionnaire).

The protocol was approved by the internal Institutional Review Board and an informed written consent was obtained from each men.

Sperm analysis

Two semen samples (7-10 days apart) were collected by masturbation after 3-5 days of sexual abstinence. After liquefaction, they were analyzed according to the World Health Organization criteria (34). The remaining spermatozoa were used for flow cytometry analysis.

Measurement of serum hormone concentrations

The hormone assays were performed by electrochemiluminescence with a Hitachi-Roche device (Cobas 6000, Roche Diagnostics, Indianapolis, IN, USA). The reference intervals were as follows: TSH= 0.3-4.2 mUI ml–1, luteinizing hormone (LH)= 1.6-9.0 mUI ml–1, follicle stimulating hormone (FSH)= 2.0-12.0 mUI ml–1, 17β-estradiol= 8.0-43.0 pg/ml, total testosterone= 2.8-8.0 ng ml–1, prolactin= 4.0-15.0 ng ml–1.

Ultrasound evaluation

The testicular and epididymal regions were carefully assessed by scrotal ultrasound using a 7.5 MHz linear transducer. The prostate-vesicular region was assessed by transrectal ultrasound using a 7.5 MHz biplan biconvex transducer (Esaote GPX Megas, Genoa, Italy). All patients underwent ultrasound evaluation before and after ejaculation, after sexual abstinence of 4 days.

The ultrasound parameters evaluated were: 1) Testicular volume evaluated by using the formula of the ellipsoid (35); 2) Cranio-caudal diameter of the epididymal head; 3) Cranio-caudal diameter of the epididymal tail; 4) Prostate volume measured by using the planimetric method (36-38). The three maximum diameters (lateral-lateral, anterior-posterior and longitudinal) of the prostate were calculated and prostate volume was expressed using the mathematical formula of the ellipsoid (diameter 1 × diameter 2 × diameter 3 × 4/3 × π); 5) Anterior-posterior diameter of the body of seminal vesicles.

Sperm flow cytometry evaluation

Flow cytometry was performed using the flow cytometer EPICS XL (Coulter Electronics, IL, Italy), as previously reported (39) to evaluate sperm mitochondrial function (after 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-benzimidazolylcarbocyanine chloride, JC-1, staining), phosphatidylserine (PS) externalization (following annexin V/propidium iodide (PI) double staining), chromatin compactness (following PI staining), and DNA fragmentation (using the TUNEL assay).

JC-1 staining: Mitochondrial membrane potential (MMP) was evaluated by staining with JC-1 (Space Import-Export, Milan, Italy), as previously reported (39). Briefly, the sperm suspension was adjusted to a density of 0.5-1 × 10^6 cells/ml and incubated with JC-1 for 10-15 min at 37 C in the dark.

Annexin V/PI assay: Staining with annexin V/PI was performed using a commercially available kit (Annexin V-FITC Apoptosis detection kit, Beckman Coulter, IL), as previously reported (39). Briefly, an aliquot containing 0.5 × 10^6 spermatozoa/ml was resuspended in 0.5 ml of binding buffer, labelled with 1 µl of annexin V-FITC plus 5 µl of PI, incubated for 10 min in the dark, and immediately analyzed. Signals were detected through FL-1 (FITC) and FL-3 (PI) detectors. The different labeling patterns in the bivariate PI/annexin V analysis identified different cell pop-

Table 1 - *Clinical, hormonal and ultrasound characteristics of the sample and the four subgroups.*

Parameter	Group A no use (no.=10)	Group B <2 h/day (no.=16)	Group C 2-4 h/day (no.=17)	Group D >4 h/day (no.=20)
Age (yr)	29.0±6.0	27.5±5.5	30.0±5.0	28.5±4.0
BMI (kg/m^2)	21.0±3.0	23.5±2.0	22.0±3.0	22.5±2.5
FSH (mUI ml–1)	4.0±2.0	3.6±2.2	5.2±2.2	3.6±2.6
LH (mUI ml–1)	5.5±2.0	4.5±2.2	5.2±1.3	4.7±2.0
Total testosterone (ng ml–1)	6.6±1.6	6.3±2.0	7.0±1.4	6.7±1.2
17β-estradiol (pg/ml)	30.2±6.0	26.2±5.5	25.0±12.0	27.5±8.5
Prolactin (ng ml–1)	12.2±6.0	14.5±5.5	11.0±6.0	14.5±4.0
TSH (mUI ml–1)	2.2±1.2	2.4±1.6	1.9±1.3	1.7±2.0
Testicular volume (ml)	22.5±3.4	21.8±4.2	24.2±2.6	22.3±4.2
Cranio-caudal diameter of the epididymal head (mm)	11.2±0.6	10.5±1.2	11.0±0.5	10.8±1.0
Cranio-caudal diameter of the epididymal tail (mm)	4.6±0.6	5.2±0.3	5.3±0.6	4.9±1.0
Prostate volume (ml)	26.6±4.5	24.8±5.6	25.0±7.0	24.2±8.0
Anterior-posterior diameter of the body of the seminal vesicles (mm)	9.2±3.0	10.6±2.3	8.8±2.2	9.5±2.7

BMI: body mass index; FSH: follicle stimulating hormone; LH: luteinizing hormone;TSH: thyrotropin.

Table 2 - *Conventional and biofunctional sperm parameters in the four examined groups.*

	Group A no use (no.=10)	Group B <2 h/day (no.=16)	Group C 2-4 h/day (no.=17)	Group D >4 h/day (no.=20)	Trousers users (no.=12)	Shirt users (no.=8)
Conventional sperm parameters						
Volume (ml)	3.0±1.2	2.8±1.4	3.0±1.1	2.9±1.5	2.8±1.3	3.0±0.8
Sperm density (10^6/ml)	71.0±7.3	68.5±6.0	72.0±6.0	67.0±11.0	65.0±5.0	68.5±8.0
Total sperm count (10^6/ejaculate)	213.0±8.8	191.8±8.4	216.0±6.6	194.3±16.5	182.8±6.5	205.5±6.4
Progressive motility (%)	46.0±3.0	42.5±6.0	41.0±6.0	44.0±3.0	42.0±2.0	44.0±6.0
Normal forms (%)	11.0±6.0	10.0±5.0	12.0±4.0	10.0±7.0	9.0±7.0	11.0±5.0
White blood cells (10^6/ml)	0.64±0.09	0.77±0.06	0.60±0.03	0.55±0.05	0.33±0.03	0.60±0.06
Biofunctional sperm parameters						
PS externalization (%)	3.5±3.0	4.1±2.0	3.9±1.8	4.3±2.5	4.8±1.6	3.9±1.8
Abnomal chromatin compactness (%)	17.0±3.0	16.7±2.6	18.0±6.0	18.8±3.0	18.8±3.0	17.2±6.0
DNA fragmentation (%)	3.0±1.2	3.2±1.6	3.1±2.2	6.6±2.2*	6.7±1.8^	5.1±1.3
Altered (low) membrane mitochondrial potential (%)	5.0±1.5	4.4±2.0	4.6±4.0	4.2±3.0	4.6±3.0	4.0±2.0

*$p<0.05$ *vs* other groups; ^$p<0.05$ *vs* shirt users.

ulations: annexin negative and PI negative were designated as alive cells; annexin positive and PI negative as PS externalized spermatozoa (early apoptotic cells).

PI staining: Sperm PI staining was performed as previously reported (39). Briefly, semen samples were centrifuged at 500 g for 10 min at room temperature, the supernatant removed and spermatozoa collected. An aliquot of about 1 × 10^6 spermatozoa was incubated in LPR DNA-Prep Reagent containing 0.1% potassium cyanide, 0.1% NaN3, non ionic detergents, salts and stabilizing (Beckman Coulter, IL, Milan, Italy) in the dark, at room temperature for 10 min and then were incubated in Stein DNA-Prep Reagent containing 50 µg/ml of PI (<0.5%), RNAsi type A (4 Kunits/ml), <0.1% NaN3, salts and stabilizing (Beckman Coulter, IL) in the dark, at room temperature for 30 min.

TUNEL assay: TUNEL assay was carried out using the Apoptosis Mebstain kit (Beckman Coulter, IL, Milan, Italy), as previously reported (39). The negative control was obtained by not adding TdT at the reaction mixture; the positive control was obtained by pre-treating spermatozoa with 1 µg/ml of RNAse-free deoxyribonuclease I (Sigma Chemical) at 37 C for 60 min before labeling. The debris was eliminated following the same procedure described above.

Statistical analysis

Results are reported as mean±SEM thoughout the study. Conventional sperm parameters were submitted to statistical analysis as the mean of the two determinations obtained from each men enrolled. The data were analyzed by 1-way analysis of variance (ANOVA) followed by the Duncan Multiple Range test. Correlation analysis was conducted by Pearson correlation test. The software SPSS 9.0 for Windows was used for statistical evaluation (SPSS Inc., Chicago IL, USA). A statistically significant difference was accepted when the *p*-value was lower than 0.05.

RESULTS

Among the examined groups were not statistically significant differences in age and BMI as well as in the group D between trousers users and shirt users (Table 2). Seminal fluid volume, sperm density, sperm total count, sperm morphology and sperm progressive motility did not show any significant variation among the examined groups, as well as any of the other conventional sperm parameters (Table 1). None of the selected ultrasound parameters (testicular and prostate volume, cranio-caudal diameter of the cephalic and caudal portion of the epididymis, anteroposterior diameter of the seminal vesicles) were significantly different between the examined groups (Table 1). Relatively to the biofunctional sperm parameters: the percentage of spermatozoa with low MMP, the percentage of spermatozoa with PS externalization, and the spermatozoa with decondensed chromatin did not show any significant variation among the examined groups (Table 2).

The percentage of spermatozoa with fragmented DNA was significantly higher only in group D compared to the other groups, and in group D the percentage of spermatozoa with fragmented DNA was significantly higher in trousers users compared to the shirt users (Table 2).

The correlation analysis showed that the percentage of spermatozoa with fragmented DNA correlated positively with the duration of use of the mobile phone (Table 3). Finally, none of the examined ultrasound parameters showed a significant correlation with the duration of use of the mobile phone (Table 3).

Table 3 - *Correlation analysis between duration of use of the mobile phone and conventional and biofunctional sperm parameters.*

Parameter	r	*p*
Sperm density (mil/ml)	–0.20	ns
Total sperm count (mil/ejaculate)	–0.19	ns
Progressive motility (%)	–0.22	ns
Normal forms (%)	–0.30	ns
Low mitochondrial membrane potential (%)	0.42	ns
Alive spermatozoa (%)	–0.33	ns
Phosphatidylserine externalized spermatozoa (%)	0.23	ns
Spermatozoa with abnormal chromatin (%)	0.22	ns
DNA fragmentation (%)	0.82	<0.001

ns: not significant.

DISCUSSION

Our clinical study evaluated the conventional sperm parameters and some of the main biofunctional sperm parameters of a series of healthy men, suggesting that the fragmentation of sperm DNA could represent the only parameter significantly altered in the subjects who use the mobile phone for more than 4 h a day and in particular for those who use the device in the pocket of the trousers. The study was carried out on healthy fertile men, without other risk factors for male infertility, with an age range between 18 and 35 yr, non-smokers, without difference between groups for age and BMI. Moreover, all the examined subjects used the same device and were classified according to the duration and mode of use of the mobile phone. Also from the ultrasound point of view, the examined patients showed no significant differences in the testicular volume (expression of testicular function) and the other ultrasound parameters of the prostate, seminal vesicles and epididymis, that are frequently altered in the chronic inflammatory process (38). In our opinion, this aspect is very important for the correct selection of the patients.

Several studies have addressed the link between mobile phones and the potential consequences on male fertility. In particular, previous experimental experiences have highlighted the following evidences: a reduction of testosterone, an increase in caspase-3, the overproduction of reactive oxygen species (5), a significant decrease in protein kinase C (6) and a decrease of the antioxidant enzymes glutathione peroxidase and superoxide dismutase (7). Another study showed a significant drop in fructose levels (9). In addition, RF-EMR exposure is associated with an increase in lipid peroxidation in the testis and epididymis (10). Moreover, the histological examination showed also a significant decrease in the diameter of seminiferous tubules in the phone group *vs* the controls (11). Finally, a significant genotoxic effect on epididymal spermatozoa was demonstrated (12).

From the clinical point of view, a significant difference was observed in sperm morphology between mobile phone users and non users. Moreover, the same study showed higher T levels and lower LH levels in the mobile phone users (13). In another clinical study, samples exposed to RF-EMW showed a significant decrease in sperm motility and viability, increase in ROS level, and decrease in ROS-TAC score (15). In step with increasing SAR, motility and vitality are significantly reduced after RF-EMR exposure, while the mitochondrial generation of reactive oxygen species and DNA fragmentation are significantly elevated. Furthermore, there is an highly significant relationship between SAR, the oxidative DNA damage bio-marker, 8-OH-dG, and DNA fragmentation after RF-EMR exposure (17).

The sperm DNA fragmentation is the presence of breaks or damage in the genetic material of the spermatozoa. Possible causes are considered the defects of maturation (40, 41), the apoptosis of the spermatozoa (42) and the oxidative stress (43). The sperm DNA fragmentation can be aggravated by altered mechanism of cellular repair or environmental insults such as hyperthermia (44).

Recently, other studies have analyzed the effects of the use of the mobile phone on sperm DNA quality, with different study designs and conflicting results. In the experimental study of Aitken et al., mice were exposed to 900 MHz radio-frequency electromagnetic radiation (RF-EMR) and after exposure, DNA damage to caudal epididymal spermatozoa was assessed and a detailed analysis of DNA integrity using QPCR (quantitative PCR) revealed statistically significant damage to both the mitochondrial genome and the nuclear beta-globin locus (12). In a subsequent study, Agarwal et al. examined the sperm DNA damage of neat semen samples [one experimental aliquot was exposed to cellular phone radiation (in talk mode), and the unexposed second aliquot served as the control] showing no significant differences from the unexposed group (15). In another study, De Iuliis et al. evaluated purified human spermatozoa exposed to RF-EMR, showing that in step with increasing SAR (specific absorption rates), the mitochondrial generation of reactive oxygen species and DNA fragmentation were significantly elevated. Moreover, it was also observed a highly significant relationship between SAR, the oxidative DNA damage bio-marker, 8-OH-dG, and DNA fragmentation (17). More recently, Falzone et al. showed that the mobile phone radiation had no statistically significant effect on any of the sperm parameters examined. In particular in this study, ejaculated, density-purified, highly motile human spermatozoa were exposed to mobile phone radiation at SAR of 2.0 and 5.7 W/kg and, at various times after exposure, the following parameters were examined: caspase 3 activity, externalization of PS, induction of DNA strand breaks, and generation of reactive oxygen species (14).

Finally, another similar study of Agarwal et al. (16) shows that, among the users >4 h/day, an alteration of the conventional sperm parameters is observed; in particular sperm motility, viability, and normal morphology appear significantly different in cell phone user groups within two sperm count groups. However, even this study does not appear comparable for the following reasons: a) infertile patients were examined; b) the biofunctional sperm parameters were not examined; c) it did not compare the different modalities of use of the mobile phone (trousers or shirt).

The results of the different studies are difficult to compare owing to the different methods used. However, none of the other studies selected the patients from the clinical point of view as in the present study, focusing on the healthy and fertile subjects users of the same device. In our opinion, this aspect is a strong point of the present study compared to other studies. Moreover, the results of this study, in agreement with the recent study of Falzone, suggest that apoptosis is not associated with sperm DNA damage. However, the present study has limitations: the small number of examined subjects and the lack of explanation of the mechanism responsible for the apparent damage of sperm DNA, that deserves further study.

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