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Effect of Wi-Fi Radiations on Sperms in Vitro: Sperm DNA Fragmentation and ROS

[1]Omkar Pokharkar, [2]Himanshu Patel, [3]Vidisha Bhatt

[1]Stem Cure Pvt Ltd, Center for reproductive medicine and stem cell development, Ahmedabad, Gujarat, India- 380013.
[2]D.Y. Patil University, school of biotechnology and bioinformatics, C.B.D Belapur, Navi Mumbai, – 400614.

Abstract: **All around the globe, studies are conducted to determine the effect of Wi-Fi on the quality of sperms both in vivo and in vitro. To determine the level of damage to the sperms in vitro, semen analysis, sperm chromatin dispersion test and ROS test was performed to map the motility, vitality, free radicals and also the fragmentation in the sperm samples which are exposed and which are not exposed to radiations emitted by Wi-Fi router. It was found after exposing sperm samples to Wi-Fi for long durations in a closed cabin placing sperm samples near Wi-Fi router can affect sperm quality to a great extent, reducing motility and vitality of sperms and causing high DNA fragmentations in sperms. On the other hand un-exposed samples kept far away from Wi-Fi source were in better condition both in terms of mobility, vitality and had fewer fragmentations. ROS test conducted on the core/raw semen samples revealed that the samples exposed for 180 minutes showed highest levels of oxidative stress displaying dark purple color in the ROS Agarose-N GEL tubes and samples subjected to Wi-Fi for 90 minutes showed high levels of oxidative stress displaying purple color and samples un-exposed to radiations showed low levels of oxidative stress and displayed light pink color. This study proved that sperm samples exposed to a Wi-Fi router connected to laptop or computer for long time causes devastating effects on sperms in vitro.**

Keywords: **Sperms, in vitro, DNA fragmentation, ROS, motility, vitality, Wi-Fi radiations, router, laptop, computer.**

I. INTRODUCTION

Wi-Fi radiations from laptops and mobiles can impair or damage sperms. Motility of the sperms are said to be reduced due to prolong exposure of sperms to radiations emitted by Wi-Fi (Wireless fidelity). This study was carried out to verify that radiations affect sperm motility and damages the DNA causing fragmentation. This experiment is to note the motility, vitality and levels of DNA damage after exposing sperm samples to Wi-Fi for certain period of time. DNA fragmentation is a way to accurately map the sperms with fragmented and non-fragmented DNA. Sperms with fragmented DNA disperse no halos and sperms with non-fragmented DNA disperse big halos and degrading sperms show small halos. If the radiation from Wi-Fi affects motility, vitality and DNA of spermatozoa it would be revealed after performing standard semen analysis according to the criteria set by WHO (World Health Organization., 2010) and Sperm chromatin dispersion test (SCD). ROS test also known as reactive oxygen species test is a test to map the oxidative stress in the sperm samples, which testifies that fragmented sperm DNA are present in the samples exposed to radiations.

II. MATERIALS AND METHODS

For this type of study semen samples from 12 fertile men with no recent history of illness aged 22-29 were obtained in sterile wide mouthed collecting jars during the period of sexual abstinence of 3 days. Each of the 12 Sperm samples were washed by swim up method which has high rate of success in obtaining viable sperms, for removal of debris and dead or immobile sperms and only motile sperms were used. This was to make sure that before exposing the samples to Wi-Fi there were all viable sperms with good motility and no pre dead or immobile sperms were present to map accurate changes due to radiations. Each of the 12 Sperm samples were divided in 3 aliquots of 0.5 ml each and out of three aliquot, 2 aliquot were exposed to Wi-Fi radiations for different periods of time, 1st aliquot was exposed to radiation for 1½ hour (90minutes) and second aliquot was exposed for 3 hours(180 minutes). These 2 aliquot were labeled as test and one aliquot was considered as control sample and was kept in different room to avoid any radiations or other factors

which would influence sperms. Semen analysis was carried out after exposure of Wi-Fi radiations to sperm samples, the motility and vitality was observed and results were recorded. The samples were exposed to radiations by keeping the samples in a closed cabin near the router of the Wi-Fi and several computers were connected to that router downloading and uploading the data to max out the radiations. The distance between samples and Wi-Fi source was about 2 inches. Unexposed samples which were placed away from radiation zone were also subjected to semen analysis and the outcomes were saved to compare and analyze the difference in the quality of both exposed and un-exposed samples. Motility percentage was calculated by using a formula: 100 X (number of motile spermatozoa)/ (total number of spermatozoa counted). Similarly, vitality was also calculated. More than 500 spermatozoa per ejaculate were evaluated for estimation of motility and vitality.

ROS (reactive oxygen species) test:

Along with washed samples of test and control, 2 aliquot (test) of core/raw samples of 0.5 ml each was also placed in the Wi-Fi zone for 90 minutes and 180 minutes respectively and control core/raw sample of 0.5ml was kept far away from Wi-Fi zone. After incubation of both control and test samples, Ros test was conducted by incubating the Agarose N-Gel tubes in boiling water at 90-100ºC for 2 minutes to melt the gel. The tubes were cooled down for 2 minutes at room temperature before addition of the sample. 0.2 ml of semen sample was added and mixed thoroughly with the melted agarose gel and air bubbles were avoided. The tubes were then placed in an incubator at 37ºC for 60 minutes. After incubation the color changes were observed immediately and were compared with the color code provided in the ROS kit to determine the level of oxidative stress present in the sample. The results of this test were noted.

DNA fragmentation test (SCD):

Then DNA fragmentation or Sperm chromatin dispersion test was carried out on all samples to check the percentage of DNA fragmentations in samples exposed for 90 minutes (1½ hour), samples exposed for 180 minutes (3 hours) and unexposed samples. The solidified agarose gel tubes were boiled in water using the float at about 90 ^{0}C – 100 ^{0}C for 2 minutes so that the gel in the tube liquefies and tubes were cooled down at 37^{0}C for 5 minutes. Then 40 µ liter of semen sample from control samples was added and mixed with liquefied agarose gel tube, similarly 40 µ liter of semen sample from first test sample (Wi-Fi for 90 minutes) was obtained and was mixed with second agarose gel eppendroff. Again 40 µ liter of sample from second test sample (Wi-Fi for 180 minutes) was extracted and mixed with third agarose gel eppendroff. These 3 tubes represent the control and test sample suspensions respectively. Three pre coated slides were used to study and compare between 1 control sample (not exposed to Wi-Fi) and 2 test samples with various periods of exposure to radiations. Then 150 µ liter of suspension from control tube was obtained with micropipette and placed on the coated slide and was covered with a cover slip. Similarly 150 µ liter of suspension from first test sample was obtained with micropipette and placed on second slide and was covered with cover slip. Again 150 µ liter of suspension from second test sample was obtained and placed on the third coated slide. These 3 slides were prepared simultaneously, air bubbles were avoided and the slides were transferred to a fridge to maintain the temperature around 4^{0}C–8^{0}C for 5 minutes. This step helps in solidification of gel on the slide. Then after 5 minutes, slides were recovered from fridge and the cover slips from the 3 slides were removed carefully such that gel integrity is not affected. Then the slides were placed on even surface and was overlaid with 1 ml of acid denaturant each and was incubated at 22 ^{0}C for about 7 minutes and the solution was drained completely after 7 minutes. Then 1 ml of lysis solution was overlaid each on all 3 slides and was incubated for 20 minutes at room temperature. After 20 minutes the lysis solution was drained completely. Then all 3 slides were washed in slanting position with 20 ml distilled water with help of syringe or a dropper. In the next step all the 3 slides were sequentially dehydrated using dehydrating solution 1, 2, and 3 provided in the kit. Then the slides were allowed to air dry for few minutes. In this time period working stain was prepared using stain solution and stain dilution buffer. Working Stain was prepared by taking 400 µ liter of stain solution and mixing it with 100 µ liter of stain dilution buffer in a dilution tube. So for 3 slides the stain was prepared 3 times. This working stain must be used within 1 hour of preparation. After air drying all 3 slides, 200 -300 µ liter of working stain was overlaid each on all 3 slides representing control and test slides respectively. Then the slides were rocked by tilting in to and fro directions for 3 minutes to maintain even distribution of stain over the slide. After 3 minutes the slides were washed by dipping and moving in a couplin jar or a beaker filled with tap water. Then the slides were kept in slanting position to air dry. This marks the end to the procedure for making sperm DNA fragmentation slides of both control and test samples. Sperm DNA fragmentation was calculated by formula: 100 X (Number of spermatozoa with fragmented DNA)/ (Total number of spermatozoa counted). More than 550 spermatozoa per ejaculate were evaluated for estimation of sperm DNA fragmentation. This entire procedure/method was performed on all 12 samples. This study took around 21 days for completion (19th January – 8th February, 2015).

III. RESULTS

By performing standard Semen analysis and sperm chromatin dispersion test, results were obtained for motility, vitality and DNA fragmentation for both exposed and un-exposed sperm samples. All results displayed below represents the decline in sperm parameters due to Wi-Fi exposure expressed through charts [see Chart I, page (28), Chart II, page(28), Chart III, page(29)]. These graphical charts reveal that, the greater the time of exposure to Wi-Fi, higher will be the DNA fragmentation % and lower will be the motility and vitality %. Average percentages for each parameter (motility, vitality and DNA fragmentation) obtained for all 12 samples were calculated and used in charts to visualize the damage caused to sperms in vitro. The difference between the qualities of samples un-exposed and exposed to Wi-Fi radiations is quite evident.

Chart I. Motility

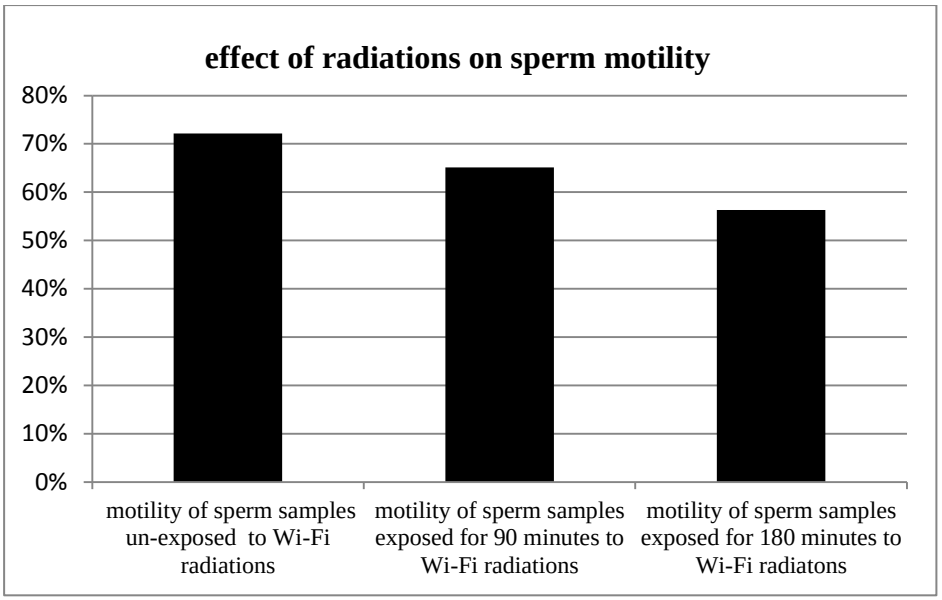
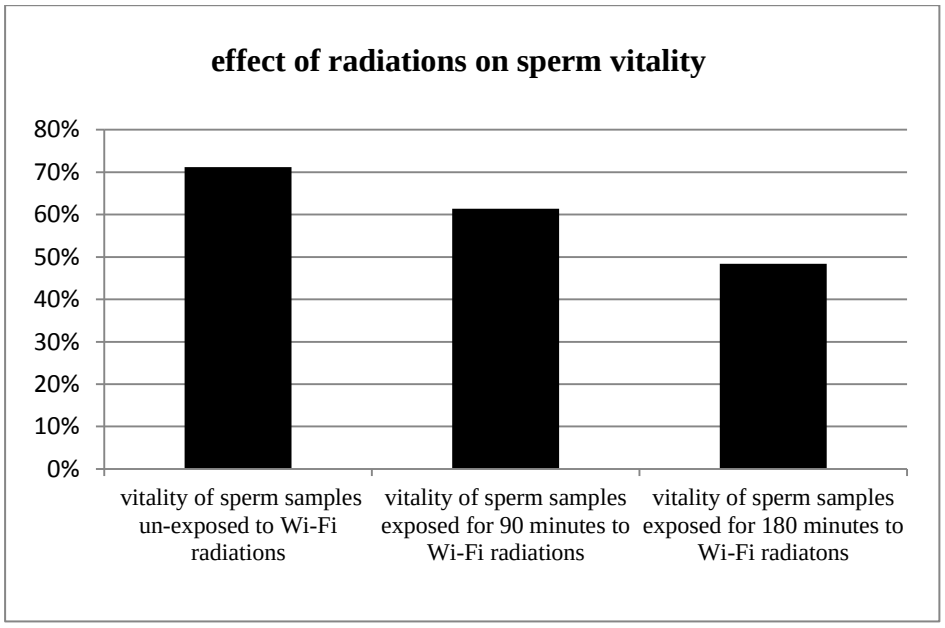
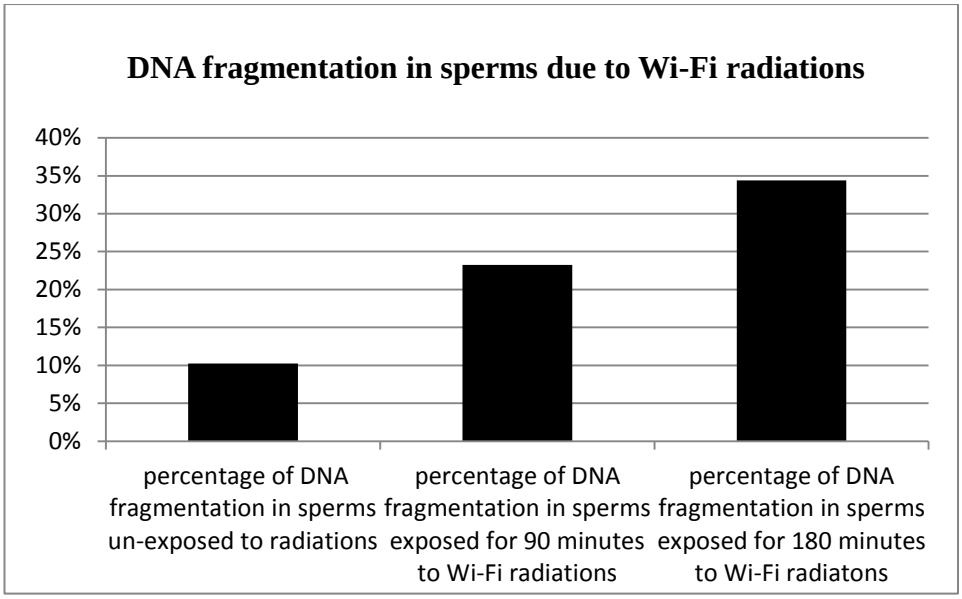


Chart II. Vitality



DNA fragmentation test also known as sperm chromatin dispersion test was carried out on all 12 samples and the slides were subjected to bright field microscopy, it was revealed that the samples exposed to Wi-Fi radiations showed high percentages of DNA fragmentation. On the other hand, Sperm samples un-exposed to Wi-Fi radiations showed fewer fragmentations.

Chart III. DNA fragmentation



Results of ROS test:

ROS test were performed using freshly collected semen samples. 1 test aliquot of core samples exposed for 90 minutes produced purple colour and the 2nd test aliquot exposed for 180 minutes produced dark purple colour. On the other hand control aliquot un-exposed to radiations showed light pink colour.

Table I. Color code for interpretation of ROS results

Reactive oxygen species(ROS)	color	Color code
Highest level of ROS in samples (180 minutes).	Dark purple	
High levels of ROS in samples (90 minutes).	purple	
Low levels of ROS in samples (un-exposed samples).	light pink	

Dark purple color was obtained for the test sample exposed for 180 minutes which indicated that highest level of oxidative stress was induced in the sample due to radiations. Whereas samples exposed for 90 minutes showed high level of oxidative stress producing purple color and the control sample which was un-exposed to radiations showed low levels of oxidative stress producing light pink color. ROS test was performed on all 12 samples for surety.

IV. CONCLUSION

From the study conducted above it was discovered that Wi-Fi radiations had detrimental effects on sperm motility and vitality. Semen analysis of 12 samples proved that the sperm motility and vitality was greatly reduced as the time of exposure to Wi-Fi radiations was increased. Analysis and calculations of DNA fragmentation caused by radiations revealed that the percentage of fragmentation also increased with increase in time of exposure to Wi-Fi. The principle of DNA fragmentation test lies in dispersion of a characteristic halos, which indicates the status of the spermatozoa. If a big halo is dispersed then the DNA of the sperm is not fragmented. On the other hand when small halo is dispersed by a sperm then it is on the verge of fragmentation/degradation and also when no halo is dispersed by sperm then it is a sign of fragmented DNA or the entire sperm is degraded. Maximum number of Sperms in un-exposed samples revealed big halos around their head and sperms in the exposed samples revealed small to no halos at all indicating that the radiations from the router has damaged the DNA so no halos were dispersed. Reactive oxygen species test performed on the samples

provided further evidence of fragmentations that occurred during the exposure. As the time of exposure of samples to the microwave radiations from the router increased the levels of oxidative stress in the samples also increased and displayed dark purple and purple color in the Agarose N-Gel tubes by reducing nitro blue tetrazolium. While un-exposed samples remained light pink pointing out low levels of free radicals [see Table I. Color code for interpretation of ROS results, page (29)]. So from the findings mentioned above it was clear that Wi-Fi radiations from routers connected to laptops and computers could cause damaging effects on sperms and could degrade their ability to stay mobile and viable.

V. DISCUSSION

There is a rapid progress in fields such as electronics and telecommunication, computers etc making mankind smart and advance. First programmable computer was made by Charles Babbage in 1830's. Alan Turing, during World War 2 created enigma code breaker machine known as Turing machines or computers. And later giant computer named "ENIAC" was built by John W. Mauchly and J. Presper Eckert at the University of Pennsylvania. Now in 21st century with advancement in technology, a new era of computers and laptops have emerged which are much smaller in size and are portable with wireless fidelity also known as Wi-Fi**.** It was long known that Wi-Fi radiations had the potential to impair sperm's ability to function properly and could reduce the fertility of men habituated to sit close to routers connected with laptop and computers for long periods of time and almost every day. Wi-Fi radiations are microwave radiations containing photons which has the slight potential of breaking the DNA strands in sperm cells but certainly do not have the potential to induce mutations and cause cancer. This experiment was a recreation of the situation in which sperms get exposed to radiations when an individual works sitting near the Wi-Fi router on his laptop with Wi-Fi enabled. In in vivo, effects of radiations must be much different than the results obtained when performed in vitro. The layers of testes acts as protective covering which absorbs much of the radiations and shields sperms from the devastating effects of radiations but only to some extent where as in in vitro there was not any biological protection to sperm samples and were more susceptible to the radiations. The samples placed in tubes were exposed to radiations by keeping the sample tubes in a closed cabin near the router of the Wi-Fi and several computers, laptops were connected to the router. Samples were not placed beneath the laptop to avoid getting a combined effect of heat and Wi-Fi radiations on sperms because the study was purely about radiations and not heat + radiations. However, keeping samples beneath laptop with Wi-Fi would mimic a person keeping laptop on his lap. But other factors such as heat would also contribute in DNA fragmentation which was undesirable in this study. In this study, we completely eliminated the risk of other factors which would influence the results. The study was focused only on effect of radiations and the results were quite intimidating. To minimize brutal effect of Wi-Fi on sperms, the connection must be disabled or turned off when not in use to avoid unnecessary radiations and also laptops must be kept on working desks or tables during its use, avoiding the excess heat emitted by laptops which comes in close contact with testes or pelvis area.

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