Original Research Paper

Volume-7 | Issue-10 | October-2017 | ISSN - 2249-555X | IF : 4.894 | IC Value : 79.96

Environmental Science

Effect of Ultraviolet Radiation on DNA fragmentation of Human Spermatozoa

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ABSTRACT Ultraviolet radiations have shown to have deleterious effects on DNA. The present study was aimed to observe the effect of UV-A radiations on sperm DNA. 30 semen samples from normozoospermic men were collected and processed. Each harvested sample was divided into two aliquots, one control and one test. The test samples were subjected to ultraviolet radiations for an hour and then tested for DNA fragmentation. The test samples showed more number of sperms with fragmented DNA compared to control samples. The results were significant at P = 0.05 level of significance.

KEYWORDS : DNA fragmentation, human spermatozoa, ultraviolet radiation.

Introduction

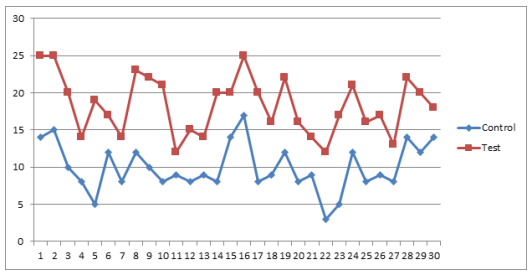
UV-A and UV-B are the radiations that reach the Earth's surface. UV radiations are known to cause the production of reactive oxygen species (ROS) in cells (Heck D et al, 2003). ROS severely affects the structure and function of DNA. Studies by Jena NR et al (2012) showed that reactive species could react with different components of DNA to produce lesions creating strand breaks (Yermilov et al. 1996; Balasubramanian et al. 1998). DNA fragmentation has shown to affect fertilization potential of human spermatozoa (Avendano C et al, 2010). The present study was aimed at observing if UV-A had any effect on DNA fragmentation rate in human spermatozoa.

Methodology

The washed semen samples were divided into two 0.1 ml aliquots, namely control and test. The test samples were subjected to UV-A radiations of 352 nm for 60 minutes, while the control samples were incubated at 37°C. After 60 minutes, the samples were mixed with 1% low melting point agar and smeared on a slide coated with 0.65% normal melting point agar. This was allowed to solidify at 4°C and then subjected to 0.8 N denaturing solution, followed by the neutralizing and lysis solution I and then the neutralizing and lysis solution II. The slides were dehydrated by serial exposure to 70%, 90%, and 100% ethanol. This was then stained for 15 minutes with the staining solution and observed under 20x for sperm chromatin dispersion around the sperm head. The sperms that did not show a pink halo had fragmented DNA.

Results and Observations

Observation graph 1 (Scale: X axis – sample serial number, Y axis – Number of sperms showing DNA fragmentation)



On being subjected to ultraviolet radiations for 60 minutes, the test samples showed an increase in the number of sperms with DNA fragmentation, which were the sperms showing no pink halo formation around the sperm head due to inability of the fragmented DNA strands to disperse. The graph clearly shows the elevated number of DNA fragmentation readings for the test samples.

Table 1: ANOVA; n = sample population

	n	Mean	Std. dev	95% CI
Control	30	9.933	3.205	(8.629, 11.237)
Test	30	18.333	3.898	(17.029, 19.637)

The control sample showed considerably lesser DNA fragmentation (mean=9.933) while the test sample showed a much higher rate of DNA fragmentation (mean=18.333). At significance level of 0.05, the P-value was 0.000, thus displaying significant difference between the control and test values.

Discussion

In the present study, it was observed that after exposure to UV-A radiations for 60 minutes, the test samples showed lesser number of sperms with a pink halo around their head compared to the control samples. This was because of the increase in fragmented DNA in the sperm head which could not disperse during the sperm chromatin dispersion test. These results were similar to the findings of Lu X Y et al (2005) who observed that UV-A and UV-B radiations were responsible for severe sperm damage in sea urchins. Pruski A et al (2009) showed similar findings in Mediterranean sea urchins where the DNA fragmentation increased 2-fold when the sperms were exposed to UV radiations. The current study similarly showed increased DNA fragmentation in sperms exposed to UV radiations. Ke W et al (1999) observed that ultraviolet radiation had a serious influence on the DNA molecular conformation damaging the hydrogen bonds and groups among the purine and pyrimidine bases in Herring sperm.

However, Catt S L et al (1997) found no damage to human sperm DNA when exposed to UV radiations. Similarly, Dietrich GJ et al (2005) observed no changes in DNA fragmentation in rainbow trout sperm.

There aren't many human studies done on the effect of UV on sperm. The current study on human sperm showed negative effect of UV radiations on DNA integrity. More studies need to be conducted in order to confirm reproducibility of the negative effects of UV on human sperm DNA.

Acknowledgement

Authors would like to thank Dr. A. S. Khemnar, Director, The Institute of Science, Mumbai and Dr. A. P. Manekar, ex Head of the Zoology Department, The Institute of Science. Sincere thanks to Fertility Clinic and IVF Centre for letting us procure discarded semen samples and use some of their equipments for the experiments.

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