Ionizing Radiation and Male Fertility

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12.1 Introduction

Exposure to ionizing radiation (IR) is becoming more common in the medical field for disease diagnoses and cancer treatment. In addition to patients undergoing treatment, IR exposure also poses a big threat to health professionals. The majority of medical investigations require radiographic testing to diagnose the disease followed by treatment, which in case of cancer patients may also require radiotherapy. Although all living creatures are at the risk of damage in response to ionizing radiation, the mammalian testes are much more sensitive to ionizing radiation. In man and in majority of animals, the testes lie outside the body and are susceptible to radiation damage (Abuelhija et al. 2013). Damage to the testes is directly proportional to the dose and time of exposure to artificial radiation or treatment. Evidence exists for sperm count reduction after treatment with low-dose testis irradiation. Moderate- to high-dose irradiation can lead to prolonged drastic decline in sperm count or even azoospermia (Abuelhija et al. 2013). The human testes appear to be more sensitive, and the recovery of spermatogenesis after radiotherapy is significantly delayed compared to most other rodents (Meistrich and Samuels 1985). This delay suggests that during the treatment period, spermatogonial stem cells become arrested at a point of their differentiation; however, the underlying mechanism of the spermatogenesis arrest and subsequent recovery in human is not known.

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This raises an important question about the post-treatment fertility of the patients and also the consequences of IR exposure on the reproductive health in medical professionals.

This chapter aims to focus on the impact of ionizing radiation on male fertility. Following a brief overview of ionizing radiation, this review will discuss selected research published on ionizing radiation, with a greater emphasis on radiotherapy and male fertility.

12.2 Ionizing Radiation

Ionizing radiation occurs in the form of an atomic or subatomic particle or an electromagnetic wave with very high kinetic energy, which has an ability to ionize the nucleus of a substance. It is believed that radioactive decay is the major reason for ionizing radiation. Electrons, protons, or neutrons are released during this process; therefore, theoretically any molecule can produce IR. However, the rate of decay differs for each molecule and can be controlled with advanced technologies. Microwaves, infrared rays, radio waves, radiant heat waves, and ultraviolet rays are generally not considered as IR. However, persistent exposure to any of the above can result into the similar effects as induced by IR (Lancranjan et al. 1975).

12.3 Sources

Classic sources of radiation which are of main concern to human beings include natural and artificial sources. Among natural sources, gamma rays from the decay products of uranium, radon gas decay products in the atmosphere, naturally occurring radionuclides, and cosmic rays from the outer space are common. A living being has daily exposure to the natural IR as they are present throughout the natural world. For example, radioactive radon gas is present in the atmosphere, and the Earth itself produces gamma rays from the decay of uranium. On an average, a person is exposed to 2.1 mSv natural radiation annually (du Plessis et al. 2014). Personnel whose work involves dealing with the general public such as teachers, physicians, and health professionals should be equipped with the basic knowledge of such radiation exposure to guide the rest of the population.

Sources of artificial radiations include radionuclides present in eating and drinking materials, X-rays used in medical diagnostic procedures, and gamma rays produced as by-products in the nuclear industry and products formed during atmospheric nuclear testing (du Plessis et al. 2014). Artificial radiation exposure can cause detrimental effects on the health of living beings. Common risk factors which can cause cancer in human beings and the impact on male fertility are summarized in Fig. 12.1. The subsequent discussion will focus on the impact of ionizing radiations on male fertility.

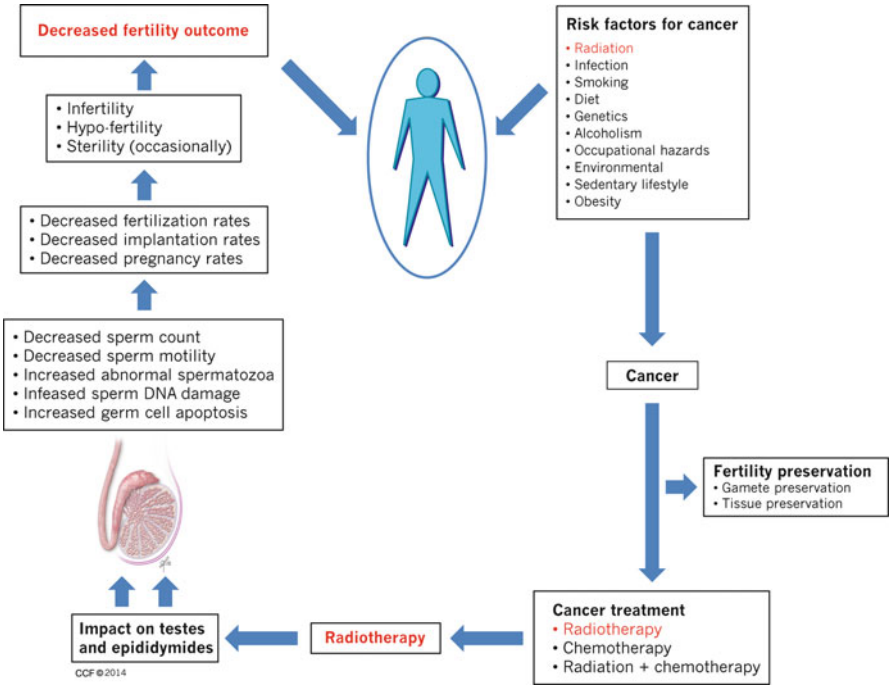

Fig. 12.1 Risk factors for cancer and impact of radiation therapy on male fertility

12.4 Radiation and Spermatogenesis

12.4.1 Animal Studies

12.4.1.1 Rodent Model

Studies conducted on different rodent models have shown that radiation have direct and dose-dependent mutagenic effects on the germ cells. High doses lead to drastically lethal effects, chromosomal aneuploidy, and point mutations (Brent 1999). In experiments, adult mouse testes were subjected to irradiation with a single shot of 1 Gy or two shots of 1 Gy with 7 days interval (Shah et al. 2009). The researchers sampled the testes every third or fourth day postirradiation to follow the recovery pattern of spermatogenesis. Treatment with a single shot of 1 Gy radiation led to a gap in spermatogenesis noted by the loss of A1 to B-spermatogonia which lasted for approximately 10 days. As expected, treatment with two shots of 1 Gy had more severe effects on germ cell elimination compared to 1 Gy. Despite germ cell elimination, spermatogenesis recovered to normal values after 6–7 weeks of treatment in both groups (Shah et al. 2009). In another study, mice were irradiated with a dose of 2 Gy, and sperm recovery pattern was observed for 10 weeks posttreatment. In this

study, spermatogenetic recovery was only 7 % at 7 weeks and reached to 84 % of the control values at 10 weeks after treatment (Searle and Beechey 1974). This shows that with a slightly higher dose of radiation, spermatogenetic recovery was much delayed. When mouse testes were irradiated with a slightly higher dose (2.4 Gy), testicular sperm count decreased significantly compared to controls at 30 days, while epididymis count decreased at 39 days after treatment (Meistrich and Samuels 1985). When using a 6 Gy dose of radiation, spermatogonial number did not recover completely but reached 90 % of the control values at 16 weeks after irradiation treatment (Erickson and Hall 1983).

Rat testis appears relatively more sensitive to irradiation damage compared to mouse testis and hence exhibits delayed spermatogenetic recovery. However, in the most widely studied and resistant strain of rats, i.e., Sprague-Dawley, the number of spermatogonia recovered to control levels within 5 weeks after 3 Gy radiation, and epididymal sperm count reached to 40 % of control after 19 weeks (Dym and Clermont 1970; Jegou et al. 1991). With treatment of 6 Gy radiations, the spermatogenetic recovery in rat was severely delayed, and 44 % of the tubules exhibited incomplete spermatogenesis even after 16 weeks of treatment (Erickson and Hall 1983). In cases of higher doses (>6 Gy), even at 26 weeks after treatment, spermatogenetic recovery was only 10 % compared to controls (Delic et al. 1986; Pinon-Lataillade et al. 1991).

12.4.1.2 Nonhuman Primates

Compared to humans, the recovery of spermatogenesis after testicular exposure to radiations is faster in rodents (Abuelhija et al. 2013). In order to have a better understanding of the process of postirradiation spermatogenic recovery, an animal model that simulates the response of human testis to radiation is needed. Nonhuman primates show much similarity to the human testis including similarities in histological stages of spermatogonia (Ehmcke and Schlatt 2006), with dramatic decrease in spermatogonial number after 2 or 4 Gy. Such decline in spermatogonial number was persistent until 6 months postirradiation, and incomplete recovery began only at 18 months of treatment (Foppiani et al. 1999; Kamischke et al. 2003). After irradiation with 0.4–0.5 Gy of X-rays, the number of A_{pale} spermatogonia (A_p) decreased to 13 % compared to control values after 11 days of treatment; however, the number of A_{dark} spermatogonia (A_d) showed no significant change at this time period but was decreased at day 14 postirradiation (van Alphen et al. 1988). Repopulation of seminiferous epithelium started from day 75 postirradiation when treated with 0.5, 1.0, and 2.0 Gy of X-rays. The number of spermatogonia (A_p and A_d) increased and reached 10 % of the control level at day 44 after treatment with 0.5 Gy dose and reached 90 % at day 200. Relatively higher doses (1.0 and 2.0 Gy) had more severe effects on spermatogonia recovery. After treatment with 1–2 Gy radiations, only 5 % of spermatogonia recovery was observed at day 44. Even at days 200 and 370 after treatment, the recovery was only 70 % (van Alphen et al. 1988). This suggests that in monkeys, the recovery of spermatogenesis is much delayed compared to rodents.

12.4.2 Human Studies

As mentioned earlier, human testes are relatively more sensitive to radiations compared to rodents. This means even subtle exposure to low-dose radiation can result in impairment of testicular function. For example, treatment with 1 Gy radiation showed significant reduction in the number of spermatocytes just after 14 day of treatment. The reduction in spermatocyte count was even more drastic at 25 days after treatment (Rowley et al. 1974). It was noted that reduction in spermatocytes count was not abrupt. Although radiation kills the cells immediately by necrosis and/or apoptosis or during their proliferation, the decline in spermatogonia number to their lowest level does not happen at once but instead occurs in a progressive manner. It may take several weeks to reach their lowest level depending upon the dose of irradiation (Rowley et al. 1974; Clifton and Bremner 1983). However, azoospermia is not achieved until 18 weeks of irradiation (Paulsen 1973). The actual reason of this gradual decline is not very clear, but one can speculate that a small population of non-cycling A-spermatogonia escapes the potential effects of radiation perhaps when they are in dormant phase of cell cycle (Meistrich 2013). Recovery of the A-spermatogonia starts after 21 weeks of irradiation which indicates that the self-renewal exceeds cell depletion at this time. As seen in animal studies, as well as in humans, the damage to spermatogenesis or testicular functions depends upon the radiation doses. High doses of radiation can result in permanent azoospermia leading to the killing of all spermatogonial stem cells. When a single dose of 10 Gy was given to the patients undergoing bone marrow transplantation, only 15 % of them regained their fertility (Meistrich 2013; Sanders et al. 1996).

The recovery of spermatogenesis after cancer treatment has been found to be linked to many factors such as the time of diagnosis, sperm parameters before the start of treatment, the nature of the treatment, both sperm parameters, and the nature of treatment, or sometimes no relation has been found with any of the above factors (Hansen et al. 1990; Petersen et al. 1999; Ishikawa et al. 2004; Bahadur et al. 2005; Pectasides et al. 2004; Eberhard et al. 2004; Lampe et al. 1997; Gandini et al. 2006). Therefore, regular cancer screening is imperative in patients with family history of cancer. This will allow the early detection and treatment of the disease with less severe effects on their future fertility.

12.5 Radiations and Sperm Parameters

12.5.1 Animal Studies

When mice were subjected to whole body irradiation of 2 Gy, a significant reduction in epididymal sperm count was observed in the irradiated group compared to the control, 28 days after treatment (Searle and Beechey 1974). This sperm concentration further reduced almost reaching zero at 42 and 49 days after treatment. Similar results in sperm count and morphology have been documented by others when

testes of two different strains of mice (C_{57} BL and B_6C_3F1) were exposed to wider range (0, 0.3, 1.0, and 3 Gy) of X-rays. The highest dose (3 Gy) caused a drastic decrease in sperm number obtained from cauda epididymis of both the strains at 42 and 49 days postirradiation. Interestingly, the rise in percentage of sperm abnormalities was notable as early as 21 days and was seen in both groups (Bruce et al. 1974). In a recent study, treatment of mice with 2 Gy dose has shown significant reduction in cauda epididymal sperm count as well as percentage sperm viability in irradiated group compared with control group even after 24 h of treatment (Li et al. 2013).

When rats were irradiated (whole body irradiation), with a wider range of irradiation doses (0.675, 1.350, 2.700, and 4.050 Gy), abnormal sperm morphology was observed compared to control values in almost all doses. These abnormalities became more severe in 2.700 and 4.050 Gy groups, where most of the sperm tails were eroded out (Chatterjee et al. 1994).

In monkeys (*Macaca fascicularis*), when testes were irradiated with a single dose of 2 Gy X-rays, mean sperm concentration per ejaculate significantly decreased to $9.2 \pm 3.5 \times 10^6$ at day 35 postirradiation compared to pretreatment value ($60.3 \pm 15.5 \times 10^6$). The decrease in sperm count was more drastic at 60 days postirradiation, and some monkeys appeared with azoospermia beyond that period (Foppiani et al. 1999). The same study found reduction in percentage sperm morphology (71.4 ± 9.9) 42 days after irradiation compared to pretreatment values (89.8 ± 3.0).

12.5.2 Human Studies

A multicenter prospective study conducted at CECOS network of France has published interesting data on radiotherapy and sperm parameters. They recruited 129 testicular germ cell tumor (TGCT) patients. One semen sample was collected from each patient before starting the treatment to serve as his control, and then treatment was started. Subsequent semen samples were collected at 3, 6, 12, and 24 months postirradiation. The results showed significant reduction in sperm count and sperm motility at 3 months postirradiation, which remained significantly low until 12 months. The sperm count and motility reached control values only after 24 months postirradiation (Bujan et al. 2013; Di Bisceglie et al. 2013). In their study, sperm count decreased at 6 months after treatment and remained low compared to baseline values up to 12 months postirradiation. Eighteen months after treatment, almost all patients recovered normal sperm count, and their counts remained unchanged for the rest of study period (36 months).

Radiation effects are more severe if it involves total body irradiation (TBI) and/or combined with cyclophosphamide (CY). Out of 25 patients who underwent a combination of bone marrow transplantation and TBI/CY treatment, only one patient had recovered spermatogenesis levels to normal. The recovery time was much delayed (75 months) compared to those patients who had only radiation therapy (Jacob et al. 1998). The kinetics of recovery of spermatogenesis after radiation

therapy is much more delayed compared to chemotherapy (Meistrich 2013; Bujan et al. 2013). In patients treated with hemi-pelvic or given pelvic radiotherapy, spermatogenic recovery did not start until 9 months postirradiation even with modest doses (0.5–0.8 Gy), and at higher dose (1.7 Gy), the delays were even more prolonged (Meistrich 2013).

12.6 Radiations and Sperm DNA

12.6.1 Animal Studies

Information on sperm DNA integrity after radiation treatment is scanty in animal models. Very few studies have been conducted on mouse. In one study, mice were subjected to acute testicular X-rays exposure with a maximum dose of 6 Gy. Spermatozoa were collected from epididymis 35 days postirradiation for DNA damage investigation. Analysis revealed that 30–40 % of spermatozoa had damaged DNA after the treatment. Some had chromosomal aneuploidy, and some others had DNA strand breaks (Pinkel et al. 1983). In an in vitro study, mouse spermatozoa recovered from epididymis were exposed to range of radiation doses (0–100 Gy). A linear dose-dependent DNA damage was observed in irradiated spermatozoa compared with controls (Haines et al. 1998). Further, in a more recent study on mice, Li et al. (2013) found a dose-dependent increase in sperm DNA fragmentation. They measured both sperm DNA fragmentation index (DFI) and high DNA stainability (HDS) through sperm chromatin structure assay (SCSA). The term DFI reflects the percentage of damaged DNA divided by the total sperm DNA which actually gives the loss of sperm DNA. HDS represents the immaturity and less condensed chromatin where sperm attain greater stainability. In these experiments, percentage of DFI was significantly higher in experimental group exposed to 2 Gy X-rays compared to control group (21.35 ± 0.78 vs 9.54 ± 0.31). Percentage HDS was also significantly higher in the experimental group compared to control group (12.03 ± 0.35 vs 3.90 ± 0.17). In another interesting study, sperm DNA damage was induced by exposing mice testicular region to different radiation doses (0.0, 2.5, 5.0, and 10.0 Gy). Comet assay was performed to quantify the sperm DNA damage. The percent tail DNA damage in spermatozoa exposed to 2.5 Gy was significantly higher compared to controls (7.98 ± 0.42 vs 5.44 ± 0.35). The difference became more significant at 5 Gy and 10 Gy (9.67 ± 0.44 and 12.14 ± 0.52) respectively (Kumar et al. 2013b).

12.6.2 Human Studies

Although sperm has highly compact DNA, it has to travel a long journey starting from testes all the way through male and female reproductive tracts before it reaches oocyte. This long passage makes it exposed to exogenous as well as endogenous threats including endogenous milieu of tracts and any foreign insult such as trauma, exposure to radiation, or toxic environment. Majority of the data available on sperm

DNA damage in cancer patients describe the effects of chemotherapy, and less is known about radiotherapy.

Some studies conducted on human sperm DNA integrity after radiotherapy report significant DNA damage compared to controls. An increase in high DNA stainability (HDS) was observed at 6 months after radiotherapy in 67 patients with pure seminoma. However, the values of sperm DNA fragmentation index (DFI) were not significantly higher than the control values (Bujan et al. 2013). Further, when patients with testicular carcinoma were treated with adjuvant radiotherapy, the DFI was significantly higher compared to the controls. The DFI was also higher compared to other treatments such as chemotherapy, which shows more drastic effects of radiation on sperm DNA. Interestingly, the increase in DFI was notable till 2 years posttreatment compared to non-treated patients (18 vs 13 %) (Stahl et al. 2004; Lord 1999).

12.7 Radiations and Sperm Fertilization Capacity

12.7.1 Animal Studies

Evidence exists that sperm can fertilize the oocyte with damaged DNA. When mice testes were irradiated with an acute dose of 2 Gy, no change in fertilization rates was observed in the irradiated group and control group at 4 weeks after treatment. However, from 4 weeks onward, the decline in fertilization rate was notable till week 7. The difference reached maximum significance at week 6 and then returned to control values after 8 weeks (Matsuda et al. 1985). In another mouse study, mice were subjected to whole body irradiation using a similar dose (2 Gy). The fertilization rates were not different in irradiated groups and the controls till 6 weeks after treatment. At weeks 7 and 8, the fertilization rates were significantly lower in irradiated groups compared to controls, and, after 9 weeks, these rates reached controls' levels (Searle and Beechey 1974). The delayed difference in fertilization rates in this study compared to the study of Matsuda and Colleagues (1985) could be because, in this earlier study, the whole body of mice was irradiated, while Matsuda et al. (1985) used acute testicular irradiation. Direct testicular irradiation may have a more severe impact on the quality of sperm compared to whole body irradiation. Interestingly, even fertilization rate was unaffected at 6 weeks in the irradiated groups (Searle and Beechey 1974); embryo implantation rate and number of live embryos were significantly lower at 6 weeks compared to controls. The difference reached the lowest value at 7 weeks and started increasing toward control values at 8 weeks (Searle and Beechey 1974). The reduction in embryo implantation rate and number of live embryos at 6 weeks in irradiated groups compared to controls reflects the damage to sperm DNA. This suggests that sperm with damaged DNA can fertilize the egg but may not support embryo development.

A recent study reported of similar results where male mice irradiated using higher doses (5–10 Gy) were mated with female mice, and the fertilization rate of

the irradiated mice did not differ from the control (Kumar et al. 2013a). However, the average number of offspring per litter was significantly reduced in irradiated groups exposed to 5 and 10 Gy (6.0 ± 0.3 and 3.3 ± 0.2 respectively) doses as compared to control group (10.1 ± 0.3). In the highest dose group (10 Gy), approximately 37 % offspring died within 10 weeks of age which shows teratogenicity of irradiation to testes (Kumar et al. 2013a).

12.7.2 Human Studies

No data is available on radiation therapy and fertilization rates in human. Scanty information is available on fertility outcome of the patients after radiation therapy used as cancer treatment. In comparison to radiation therapy, reasonable data is published on fertility outcome in patients after chemotherapy. Studies published on chemotherapy report a greater concern about fertility issues in patients after cancer treatment. A case-control American study showed that patients treated for testicular germ cell tumor were significantly more likely to experience fertility problems in terms of fathering a child compared to control population. Such cases had more consults for fertility distress as compared to the controls (Kim et al. 2010). The results of a more recent study showed that in patients with testicular tumor who attempted to conceive after radiotherapy alone or combined with chemotherapy (either naturally or through assisted reproductive techniques using fresh or frozen semen), live birth rates were as low as 32 % (9/28) and 28 % (2/7) respectively (Ping et al. 2014). The data also showed four miscarriages from the entire attempts made by 73 couples in all groups. However, there was no evidence of congenital abnormalities or any malignancies among the babies born after the treatment. The debate on the transgenerational effects of radiotherapy is going on and more data is required. Nevertheless, the deleterious effects of radiation therapy and its exposure should not be ignored, and effective protective measures must be adopted to safeguard male reproductive health.

12.8 Conclusions

Radiation therapy has negative impact on spermatogenesis and affects qualitative as well as quantitative sperm parameters in animals as well as humans. The extent to which the testes respond to radiation depends upon the dose and duration of exposure or treatment. Extreme care must be taken while treating cancer patients. Spermatozoa cryopreservation must be advised to all patients before treatment. It may be advisable to go for germ cell preservation in patients who have not achieved puberty and in whom spermatogenesis has not yet begun. In a multidisciplinary approach, patients must be counseled for the cryopreservation of gametes or tissues, and the options of assisted reproductive techniques should be well explained.

12.9 Future Directions

Although no abnormalities have been reported in children fathered by patients treated for cancer, larger prospective studies are required to represent the true data. Further, animal studies should be carried out to investigate the potential transgenerational effects of radiation therapy or male-mediated developmental toxicity. Couples should use contraceptive methods for 1–2 years after cancer treatment to avoid possible potential risk of DNA damage and aneuploidy caused by radiation therapy, which can affect embryo development.

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