Preservation of Menstrual Function in Adolescent and Young Females

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Every year thousands of young teenagers are afflicted with different types of cancer and receive gonadotoxic chemotherapy and radiotherapy regimens, causing depletion of germ cells in the gonads and premature gonadal failure. In this review, we discuss and outline the current strategies and the future directions of fertility preservation and ovarian cryopreservation and transplantation in adolescents and young females.

Key words: **ovary; chemotherapy; radiotherapy; fertility preservation; menstrual function; ovarian cryopreservation**

Introduction

Over the past 25 years, in the modern era of cancer treatment, with advanced diagnostic modalities, improved surgical technique, combination chemotherapy, radiotherapy and supportive care, there have been significant improvements in the 5-year relative survival rate for many childhood cancers. The 5-year relative survival rate among children for all cancer sites combined increased from 58% for patients diagnosed in 1975 to 1977 to 79% for those diagnosed in 1996 to 2002.[1] This increase in survival rates has given rise to a new population, *adult survivors of childhood cancer*. As a result of exposure to high cumulative doses of specific chemotherapeutic agents (e.g., alkylating agents, anthracyclines) and radiotherapy or both, many treatment-related adverse health outcomes have been identified in this newly described group of patients, ranging from metabolic and endocrine abnormalities to cognitive function deficits.[2] Gonadal failure and subsequent poor long-term reproductive outcomes are other important sequels of previous exposure to chemo- and/or radiotherapy during childhood. An accelerated and premature depletion of germ cells in the gonads of both sexes induced by chemo- and radiotherapy is the main mechanism underlying gonadal failure. Furthermore, in fertile survivors of childhood cancers who were exposed to pelvic or spinal radiotherapy during childhood, there is increased risk of early pregnancy loss, preterm birth, and delivery of low- or very-low-birthweight infants because of the impact of radiotherapy on the uterus and pelvic structures.[3]

It also should be remembered that besides malignancies, treatment of certain pre-cancerous and benign conditions such as myelodysplasia, aplastic anemia, and systemic lupus erythematosus may necessitate administration of high-dose chemotherapeutics with and without stem cell transplantation.[4] Additionally, some patients with an abnormal chromosomal karyotype such as Turner syndrome will eventually develop premature ovarian failure as a result of accelerated atresia of germ cells in the ovary. Therefore, as can be seen easily, concerns about current and future fertility after chemo- and radiotherapy should not just be limited to cancer patients, but should also be extended to other patients whose fertility is jeopardized by the chemotherapy required for the treatment for their benign illnesses; all these patients need to be counseled accordingly. In this review, we discuss the needs and outline the current fertility preservation strategies in adolescent and young female patients who are at risk for gonadal failure.

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Chemotherapy and Radiation-Induced Ovarian Damage in Female Adolescents: Reproductive Outcomes of Survivors of Childhood Cancer

There are numerous treatment-related risks for long-term adverse outcomes among survivors of childhood cancer after exposure to chemotherapeutic drugs such as alkylating agents and anthracyclines, radiotherapy, and surgery. It appears that female sex is more commonly associated with higher treatment-related risks such as cognitive dysfunction after cranial irradiation, poor cardiovascular outcomes, obesity, radiation-associated differences in pubertal timing, development of primary hypothyroidism, breast cancer as a second malignant neoplasm, and osteonecrosis.[2]

Since the first report demonstrating toxic effects of nitrogen mustard on the testis appeared in 1948,[5] numerous studies have demonstrated the harmful effects of chemo- and radiotherapy on the gonads of both sexes. Abdominal, pelvic, and spinal radiotherapy and certain chemotherapeutic drugs, especially alkylating agents, have been shown to increase the risk of ovarian failure in female cancer survivors.[4,6–11]

If the loss of ovarian function develops during or shortly after the completion of cancer therapy, it is termed acute ovarian failure (AOF). Even though its precise incidence is not known, and data concerning its risk factors are limited, AOF is known to develop at least in a subset of survivors of pediatric and adolescent cancers. One study that included 3390 female participants from the Childhood Cancer Survivor Study who were greater than 18 years of age showed that 215 patients (6.3%) developed AOF. Survivors with AOF were older at diagnosis and more likely to have been diagnosed with Hodgkin's lymphoma or to have received abdominal or pelvic radiotherapy than survivors without AOF. Among survivors with AOF, 116 (54%) had received at least 1000-cGy ovarian irradiation. Increasing doses of ovarian irradiation, exposure to procarbazine, and exposure to cyclophosphamide at ages 13–20 years were found to be independent risk factors for AOF.[12]

Another form of gonadal failure is the development of premature ovarian failure in childhood cancer survivors who retained ovarian function after completion of cancer treatment. In this group of patients the loss of ovarian function occurs years after completion of cancer therapy after a window of normal functioning. A recent study analyzed ovarian function in 2819 survivors of childhood cancer who were diagnosed at median age 7 and followed-up for as long as 40 years of age with 1065 sibling controls. The cumulative incidence of nonsurgical premature menopause was higher for survivors than for siblings (8% versus 0.8%). Identified risk factors include attained age, exposure to increasing doses of radiation to the ovaries, increasing alkylating agent score (based on number of alkylating agents and cumulative dose), and a diagnosis of Hodgkin's lymphoma. For survivors who were treated with alkylating agents plus abdominopelvic radiation, the cumulative incidence of nonsurgical premature menopause approached 30%.[13]

Nevertheless, nearly the entire body of information on the impact of cancer treatments on human fertility is based on the crude assessment of menstrual function, which is not a sensitive marker of fertility.[14] In one of our recent papers, for the first time we provided quantitative histologic evidence of chemotherapy-induced ovarian damage in humans (especially from alkylating drug regimens) by comparing primordial follicle counts in age-matched cancer patients, and we documented the impact of cancer treatments on ovarian stromal cells, which had not been studied before.[15] In order to further dissect the time course and the gonadotoxic action of mechanisms of different cancer drugs on the human ovary, which is not practically possible in a clinical setting, we developed a xenograft model.[16] That model enabled us to show the devastating effect of cyclophosphamide (an alkylating agent) on human ovary with histomorphometric and immunohistochemical analyses, avoiding the need for lengthy clinical studies to assess gonadotoxic potential of different cancer drugs. Abdominal, pelvic, or spinal irradiation is associated with increased risk of developing acute ovarian failure, especially if both ovaries are within the treatment field.[6,18] The higher the dose of radiation, the higher the risk of premature ovarian failure. A single dose is more toxic than a fractionated dose. Recent studies suggest that LD 50, the radiation dose required to kill 50% of oocytes of the human oocyte, is <2 Gy.[19] Ovaries of younger individuals are more resistant to damage from irradiation than ovaries of older individuals.[20,21] A radiation dose of 6 Gy may be sufficient to produce irreversible ovarian damage in women older than 40 years of age, in contrast with the 10- to 20-Gy doses needed to induce permanent ovarian failure in the majority of females treated during childhood.[22,23] Because younger patients harbor more primordial follicles in their ovaries, they are more likely to retain some residual ovarian function after radiotherapy than older patients. The most devastating effects of radiotherapy on the ovary occur in patients who receive a stem cell transplant with high-dose total-body irradiation (TBI). In one series, almost 100% of patients who had undergone a marrow transplant

with TBI after age 10 developed acute ovarian failure, whereas approximately 50% of girls who had received a transplant before age 10 suffered acute loss of ovarian function.[24] Total body irradiation, given as a single dose or fractionated (10–15 Gy), is often used in combination with gonadotoxic cyclophosphamide or melphalan. One study showed that all of 144 patients receiving TBI with cyclophosphamide for bone marrow transplantation (BMT) developed amenorrhea in the first 3 years. Return of menses occurred 3–7 years post transplant only in 9 patients; all were younger than age 25.[25] Another harmful effect of radiation is hypothalamic amenorrhea. The risk of gonadotropin deficiency appears to increase after doses >30 Gy to the hypothalamic–pituitary unit.[26]

Uterine function is also often compromised by radiation-induced damage to uterine vascular and muscular structures, resulting in decreased uterine blood flow, reduced uterine volume, decreased endometrial thickness, and loss of distensibility. For instance, Only four of 38 patients who had received whole-body irradiation (20–30 Gy) during childhood had documented pregnancies and all resulted in mid-trimester miscarriage.[11] Women exposed to radiation postpubertally have a larger uterus and greater likelihood of live birth than those exposed prepubertally.[27] Furthermore, women with ovarian failure secondary to whole-body irradiation (20–30 Gy) have significantly reduced uterine size with no improvement in blood flow and endometrial thickness in response to exogenous sex hormones.[28]

The report of the Childhood Cancer Survey Study states that radiation therapy is associated with lower birth weight in the offspring and a higher risk of miscarriage in childhood cancer survivors.[29] Children who received 25 Gy to the abdomen or pelvis have a higher risk for the development of pregnancy-related complications such as lower birth weight and perinatal death. Survivors' children were more likely to be born preterm than siblings' children (21.1% versus 12.6%; OR = 1.9, 95% confidence interval [CI] = 1.4 to 2.4; $P < .001$). Compared with the children of survivors who did not receive any radiotherapy, the children of survivors treated with high-dose radiotherapy to the uterus (>500 cGy) had increased risks of being born preterm (3.5 times higher), low birth weight (6.8 times higher), and small for gestational age (SGA) (4 times higher). Increased risks were also apparent at lower uterine radiotherapy doses (starting at 50 cGy for preterm birth and at 250 cGy for low birth weight). Late effects of treatment for female childhood cancer patients may include restricted fetal growth and early births among their offspring, with risks concentrated among women who receive pelvic irradiation.[13] However, relative risk of malformations among the children of cancer survivors is not significantly different from that of their siblings.[30,31]

It should be emphasized that the timing of menarche may be impaired in survivors of childhood cancer, especially in those exposed to cranial and craniospinal radiotherapy. It has been shown in 949 female survivors of acute lymphoblastic leukemia (ALL) that survivors treated with chemotherapy alone, including those exposed to alkylating agents, experienced menarche at a rate similar to that of siblings. However, compared to chemotherapy alone, cranial radiotherapy was associated with early menarche (age < 10), whereas craniospinal radiotherapy was associated with both early and late menarche. There were no differences in effect between <20 and ≥20 Gy radiotherapy doses. In multivariable analysis, younger age at diagnosis was an independent risk factor for early menarche. Few female childhood ALL survivors experienced menarche outside of the normal range. Alkylating agent exposure was not associated with abnormal timing. However, those exposed to cranial and craniospinal radiotherapy, especially at a young age, should be monitored closely for abnormal timing of menarche.[32]

Options of Fertility Preservation in Female Adolescents and Young Adults with Cancer

Oocyte Cryopreservation

Since embryo freezing is not a practical option for children, oocyte freezing may be considered in some adolescent cases. The technology is improving and our recent meta-analysis showed that live-birth rates per injected oocyte and embryo transfer, respectively, were 3.4 and 21.6% for oocytes with the slow freezing method.[33] Nevertheless ovarian stimulation and transvaginal oocyte retrieval is usually not practical in most pediatric cases.

Cryopreservation of Ovarian Tissue

Ovarian cryopreservation may be the only option for fertility preservation, especially in prepubertal children and those who do not have time to undergo ovarian stimulation for oocyte or embryo cryopreservation. In fact, the American Society of Clinical Oncology has issued recent clinical guidelines encouraging fertility preservation among all young cancer survivors with an interest in fertility.[34]

TABLE 1. Summary of fertility preservation strategies in children and young adolescents

	Strategies to preserve fertility
Reducing cancer treatment–related damage	• Eliminating alkylating agents from the regimens • Using conformal radiotherapy such as intensity-modulated radiotherapy
Protection from chemotherapy via pharmacologic agents	• Benefit of ovarian suppression by GnRHa is not proven and without proven biological basis • Sphingosine-1-phosphate: protective against both chemo- and radiotherapy in mice and primates; no data in humans
Transposition of gonads	• May offer protection in pelvic irradiation; does not eliminate scattered radiation to the gonads; potential risk of metastatic foci in the transposed gonads; more successful in males
Cryopreservation of germ cells	• Ovarian tissue freezing still experimental • Embryo freezing not feasible in children • Oocyte freezing may be possible in older children

Ovarian cortex contains primordial follicles with oocytes arrested in the diplotene of prophase of first meiotic division. It has been suggested that relatively high surface/volume ratio, low metabolic rate, and the absence of the zona pellucida make primordial follicles less susceptible to cryodamage.[4] Ovarian tissue cryopreservation and transplantation studies date back to the 1950s, but its application to human ovarian tissue is confined to the last decade.[35] Initial studies were disappointing until the discovery of effective modern cryoprotectants such as ethylene glycol, DMSO, and propanediol and the availability of automated cryopreservation machines. Glycerol was the only available cryoprotectant in 1960s, but was found ineffective for cryopreservation of human oocytes and ovarian tissue.[36] The first case of autologous ovarian transplantation with cryopreserved tissue was reported in 2000.[37] Likewise, first reports of embryo generation and spontaneous pregnancies after subcutaneous transplantation of frozen banked tissue dates back only to 2004 and 2006.[38,39] More recently two live births after autologous ovarian transplantation to pelvis have been reported, even though the origin of pregnancies could not be confirmed with 100% certainty, as, especially in the report by Donnez *et al*. the patient continued to ovulate from the *in situ* ovary.[40,41]

Ovarian tissue removal for the purpose of freezing is done by laparoscopy and appears to be complication-free even in those with complex medical problems and thrombocytopenia.[42] Fertility preservation strategies in children and young adolescents are summarized in Table 1. The indications of all ovarian cryopreservation cases in this group are shown in Table 2. Nevertheless, ovarian tissue freezing and transplantation is still experimental and the success rates await validation in clinical trials.

Table 2.

Patients	Age	Diagnosis	Indication for ovarian cryopreservation
1	4	Diamond Blackfan Syndrome	HSCT
2	6	Thalassemia major	HSCT
3	8	Diploid-Triploid Mosaicism	Gonadectomy
4	10	Acute Lymphoblastic Leukemia	HSCT
5	10	Acute Lymphoblastic Leukemia	HSCT
6	16	SLE with diffuse proliferative GN	Chemotherapy
7	17	Ovarian papillary serous carcinoma	Gonadectomy
8	17	Myelodysplasia	HSCT
9	18	Ewing Sarcoma	Chemotherapy
10	18	Hodgkin's lymphoma	HSCT
11	18	Acute Myelocytic Leukemia	HSCT

HSCT = Hematopoietic stem cell transplantation; SLE = Systemic lupus erythematosus; GN = Glomerulonephritis.

Ovarian Transposition (Oophoropexy)

Administration of spinal radiation for the treatment of acute lymphoblastic leukemia and brain tumors appears to result in clinically significant ovarian damage in some young females.[43] Girls treated

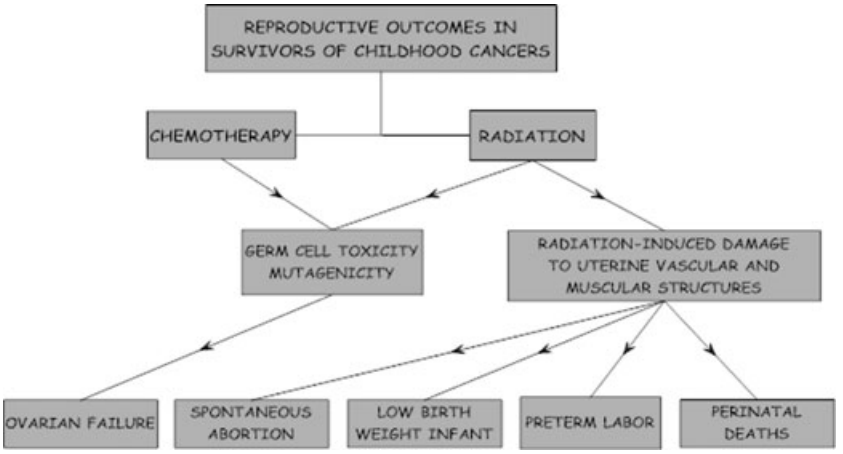

FIGURE 1. Summary of harmful effects of chemotherapy and radiation on the reproductive system.

with whole abdominal and/or pelvic irradiation for Hodgkin's disease, Wilms' tumor, or other solid tumors (e.g., rhabdomyosarcoma, neuroblastoma) are at high risk of acute ovarian failure.[44,45] When ovarian transposition is performed prior to radiotherapy, however, ovarian function is retained in the majority of young girls and adolescent females.[22,47] If the patient is to undergo abdominal surgery, ovaries can be transposed simultaneously, or, if she is to be treated nonsurgically, laparoscopic transposition can be performed before the scheduled radiotherapy. The success with fertility preservation by ovarian transposition prior to radiotherapy varies between 16 and 90%.[4] This likelihood of success is affected by the degree of scatter radiation, vascular compromise, the age of the patient, dose of radiation, whether the ovaries were shielded, whether concomitant chemotherapy is used, and whether vaginal brachytherapy or pelvic external beam irradiation plus brachytherapy were used.[4] In addition, this surgical procedure is not without complications: fallopian tube infarction, chronic ovarian pain, ovarian cyst formation, and migration of ovaries back to their original position before radiotherapy have been reported, some of which may require additional gynecologic surgery.[48] When ovaries are transposed to an abdominal position, spontaneous pregnancy may not be possible, unless a second procedure is performed to relocate ovaries back to the pelvis. In addition, should these patients need IVF in the future, oocyte retrieval may become technically more challenging. Therefore candidates for ovarian transposition should be selected carefully, accounting for all the variables that may affect its success rates. It should also be borne in mind that, when gonadotoxic chemotherapy is used along with radiation, there is no strong rationale to perform this procedure.

Another option to decrease the dose of scattered radiation to the ovaries is intensity-modulated radiotherapy (IMRT). IMRT is a new conformal radiotherapy that delivers radiation to the tumor more precisely, while sparing the surrounding tissues. Its ability to simultaneously create multiple targets and multiple avoidance structures may guide the oncologist to reduce the scattered dose to the ovary.[49]

It has been hypothesized, largely on the basis of the debated role of gonadal suppression in men in preserving testicular function against chemotherapy, and partially in the erroneous belief that prepubertal girls are not affected by gonadotoxic cancer treatment, that ovarian suppression can be protective. Even though there are some animal studies with conflicting results of the protective effect of GnRH agonists against chemotherapy and radiotherapy,[50,51] and some anecdotal reports of clinical trials done with a few patients with short-term follow-up and historical controls,[52] there is neither a reliable clinical study nor molecular evidence showing that GnRH agonists protect the human ovaries from chemotherapy and radiation.[15,53,54]

The effects of chemotherapy and radiation on reproduction with available options to preserve fertility are summarized in FIGURE 1. In conclusion, the options for fertility preservation in adolescents and young females who are at risk of developing ovarian failure are more limited than in adult females. Even though the risk of acute ovarian failure appears to develop in a minority of patients with some recovery in long term, it should be kept in mind that

there are still significant risks for poor reproductive outcomes at adulthood. Survivors of childhood cancer have 13 times the risk of premature ovarian failure compared to their siblings. In addition, if they were also exposed to pelvic/abdominal radiotherapy during childhood, their pregnancies are complicated with low-birth weight, preterm labor, spontaneous abortions, and perinatal death. Therefore every child or teenager diagnosed with cancer should be thoroughly counseled, along with their parents, about the risks for adverse reproductive outcomes in the future and their options for fertility preservation.

Conflict of Interest

The authors declare no conflicts of interest.

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