

ERİŞKİN YAŞA GELEN ÇOCUKLUK ÇAĞI VE ADOLESAN KANSER HASTALARINDA OVER FONKSİYONU VE REPRODÜKTİF SEYİR

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ÖZET

Son 25 yılda 15 yaş ve altındaki çocuklarda 5 yıllık sağ kalım sürelerinde belirgin bir iyileşme gözlenmektedir. Bu artış yeni bir insan popülasyonunun ortaya çıkışına yol açmıştır: Erişkin yaşa gelen çocukluk çağı kanser hastaları. Malesef sitotoksik kemoterapi ilaçları ve radyoterapiye maruz kalan çocuklarda peç çok metabolik ve endokrin problem izlenmektedir. Gonadal (over) yetmezlik ve erken kalıcı menopoza bu gruptaki hastalarda son yıllarda daha çok farkedilir hale gelmiştir. Dolayısıyla fertilitenin bu hastalarda korunması giderek önemi atran bir şekilde gündeme gelmektedir. Üstelik kendisi kanser olmadığı halde kanser tedavisi ve kemik iliği nakli gerektiren bazı hastalıklarda da aynı riskler mevcuttur. Turner sendromu ve galaktozemi gibi bir diğer grup hastalıkta ise erken ce hızlı folikül kaybı mutlak erken menopoza ile sonuçlanmaktadır. Görüleceği üzere fertilitenin korunması sadece kanser hastalarına sınırlı değildir. Bizde bu konuda 4 ana başlık halinde kapsamlı bir derlemeler hazırlayarak okuyucuları güncel bilgilerle aydınlatmayı hedefledik. İlk derleme çocukluk çağı kanserlerinde kullanılan tedavilerin over üzerine olan etkileri, overde germ hücre dinamiği ve over rezervi ölçüm testlerine yöneliktir. 2. derleme okuyucular kanser tedavilerinin over dışı uterin etkileri ve bunların klinik sonuçları üzerinedir. 3. Takip eden derleme ise pediatrik kanserlerin tedavilerinde kullanılan kemoterapi ve radyasyonun sebep olduğu üreme dışı endokrin etkilerine yöneliktir. Nihayet 4. derleme ise pediatrik yaş grubu kanser hastalarında fertiliteniyi koruma stratejilerini içermektedir.

Anahtar kelimeler: Fertilitenin korunması, ovarian dokusu dondurma, pediatrik kanserler, endokrin disfonksiyon, kemoterapi, radyasyon

ABSTRACT

Over the past 25 years, the 5-year relative survival rate among children under age 15 for all cancer sites significantly improved. Prolonged survival has given rise to a new population, adult survivors of childhood cancer. Unfortunately, as a result of exposure to cytotoxic chemotherapeutic agents (e.g., alkylating agents, anthracyclines) and/or radiotherapy, many adverse health conditions have emerged in the childhood cancer survivors ranging from metabolic and endocrine abnormalities to cognitive function deficits. Gonadal (ovarian) failure and other poor reproductive outcomes at long-term are now being recognized as important sequelae of previous exposure to chemo and/or radiotherapy during childhood. Therefore, preservation of gonadal function and fertility has recently emerged as an important quality of life issues for pediatric cancer patients. Furthermore, the indications for fertility preservation have also extended beyond cancer since patients with certain precancerous and benign illnesses

such as myelodysplasia, thalassemia, systemic lupus erythematosus may have to receive cytotoxic chemotherapy/radiation and stem cell transplantation for the cure. Certain disorders which are genetically predisposed to premature ovarian failure (POF) such as Turner syndrome and galactosemia are also potential candidates for fertility preservation. Therefore, we aimed in this review article to provide an update on the impact of cancer treatment on reproductive and other endocrine functions in the female survivors of childhood cancers, and to discuss the needs and outline the current fertility preservation strategies in these patients.

Keywords: Fertility preservation, ovarian tissue freezing, pediatric cancers, endocrine dysfunctions, chemotherapy, radiation

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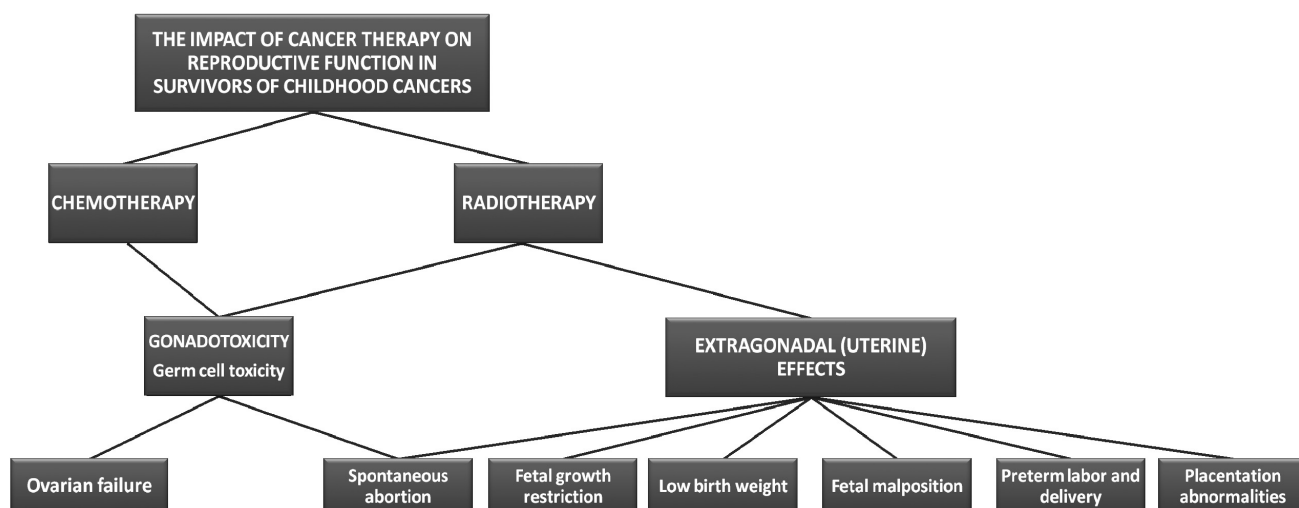
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INTRODUCTION

Cancer is the second leading cause of death among children between ages 1 to 14, surpassed only by accidents [1]. Nearly one third of the cancers diagnosed in children ages birth to 14 years are leukemias (particularly acute lymphocytic leukemia), followed by cancer of the brain and other nervous system (21%), soft tissue sarcomas (including neuroblastoma [7%] and rhabdomyosarcoma [3%]), renal (Wilms) tumors (5%), and non-Hodgkin lymphoma (4%) [1]. Over the past 25 years, the 5-year relative survival rate among children under age 15 for all cancer sites combined significantly improved from 58% for patients diagnosed during 1975–1977, to 81% for those diagnosed from 1999 to 2005 due to the employment of new and more effective targeted therapies, and chemotherapy and radiotherapy regimens and advancements in diagnostic modalities [1]. The most significant increase in the survival rate was observed in non-Hodgkin lymphoma (increased to 86% from 44%; the rate of change %42) followed by acute myeloid leukemia (41%) and acute lymphocytic leukemia (%31) [1]. Overall, Hodgkin lymphoma has the highest 5-year survival rate among all pediatric cancers (95%). Prolonged survival has given rise to a new population, *adult survivors of childhood cancer*. Unfortunately, as a result of exposure to cytotoxic chemotherapeutic agents (e.g., alkylating agents, anthracycli-

nes) and/or radiotherapy, many adverse health conditions have emerged in the childhood cancer survivors ranging from metabolic and endocrine abnormalities to cognitive function deficits [2-3]. Gonadal (ovarian) failure and other poor reproductive outcomes at long-term are now being recognized as important sequelae of previous exposure to chemo and/or radiotherapy during childhood. Cytotoxic chemotherapy regimens and radiotherapy induce apoptotic death of the follicles in the ovary leading to early exhaustion of follicle stockpile and premature ovarian failure [4]. Unfortunately, end-organ damage is not limited to the ovary. In fertile survivors of childhood cancers who were exposed to pelvic or spinal radiotherapy during childhood, there is an increased risk of early pregnancy loss, preterm birth, and delivery of low- or very-low birth weight infants due to the impact of radiotherapy on the uterus and pelvic structures [5].

Besides pediatric cancers, patients with certain precancerous and benign illnesses such as myelodysplasia, aplastic anemia, thalassemia, and systemic lupus erythematosus may have to receive high dose chemotherapy with or without stem cell transplantation for the treatment of their primary diseases (Table-1) [2]. Moreover, the indications for fertility preservation have also extended to those who are genetically predisposed to premature ovarian failure (POF). Turner syndrome and galactosemia are two striking



Figur I—The impact of chemotherapy and radiation on the reproductive system in the childhood cancer survivors. Particularly for radiation end-organ damage is not limited to the ovaries but also uterine functions are frequently compromised by radiotherapy leading to adverse pregnancy outcomes in the adult survivors of pediatric cancer patients.

Tablo 1—The Diseases Requiring Counseling for Fertility Preservation in the Children. The Indications Extend Beyond Cancer.

Disease	Indication for fertility preservation
Leukemia-Lymphoma	Chemotherapy-HSCT
Myelodysplasia	HSCT
Aplastic anemia	HSCT
Thalassemia major	HSCT
Other hematological diseases	Chemotherapy-HSCT
Autoimmune diseases	Chemotherapy-HSCT
Wegener Disease	Chemotherapy-HSCT
Systemic lupus erythematosus	Chemotherapy
Wilms tumor	Chemotherapy
Osteosarcoma	Chemotherapy
Ewing Sarcoma	Chemotherapy
Tumors of the pelvis and spine	Chemotherapy-Radiotherapy
Retroperitoneal tumors	Chemotherapy-Radiotherapy
Rhabdomyosarcoma	Radiotherapy
Turner Syndrome	Premature ovarian failure
Galactosemia	Premature ovarian failure
Fragile-X syndrome	Premature ovarian failure
Mosaicism	Gonadectomy
Teratoma	Gonadectomy

HSCT: Hematopoietic stem cell transplantation

examples. Both disorders are characterized by accelerated and premature depletion of the oocytes in the ovary, culminating in premature ovarian failure. Therefore, preservation of gonadal function and fertility has become one of the major quality of life issues for pediatric and adult cancer patients. Accordingly, clinical guidelines, encouraging fertility preservation among all young cancer survivors with interest in fertility have been issued by the American Society of Clinical Oncology [6].

THE IMPACT OF CANCER TREATMENT ON OVARIAN FUNCTION IN THE SURVIVORS OF CHILDHOOD CANCERS

Chemotherapy

Chemotherapy drugs have different cytotoxicity profiles depending upon their category as shown in the Table-2. Chemotherapeutics of alkylating category such as cyclophosphamide, busulfan and melphalan have the highest gonadotoxic potential. Unfortunately many of these alkylating drugs are included in the first line therapy of many solid and hematological malignancies commonly diagnosed in children such as Hodgkin's lymphoma [7]. The index drug cyclophosphamide is metabolized to two active metabolites in the body, phosphoramidate mustard and acrolein. While acrolein exerts its toxicity on the bladder causing hemorrhagic cystitis, phosphoramidate mustard is the main product that is responsible for the follicular damage in the ovary [8]. High-dose cyclophosphamide (200 mg/kg) is frequently used as conditioning therapy before bone marrow transplantation (BMT), either alone, or in combination with other chemotherapeutic agents or total body irradiation. A human ovarian xenograft model showed that both oocyte and somatic cells of the follicles (ie, granulosa cells) undergo apoptotic cell death after exposure to cytotoxic chemotherapeutics such as cyclophosphamide as assessed by terminal nucleotidyl transferase-mediated nick end labeling (TUNEL) assay [4]. This implies that not only oocyte but also somatic compartment of follicles are damaged by cytotoxic chemotherapy. This is in accordance with a recent finding that human ovaries exposed to cytotoxic chemotherapy regimens in vivo produce significantly less estradiol in vitro [9]. Platinum cancer drugs closely follow the alkylating category in terms of gonadotoxicity, thence are called alkylating like agents. Taxanes and anthracycline family are less toxic than alkylating agents. The members of antimetabolite group such as methotrexate and 5-fluorouracil have the least toxicity. It should also be noted that cancer drugs with moderate or minimal gonadal toxicity may induce more toxicity in the ovary if they are used for longer period of time; at higher doses; and in patients at advanced age and with poor ovarian reserve.

Table 2—Chemotherapy drugs. Chemotherapy agents are classified in the table according to their category and gonadotoxic potential.

Category	Group	Gonadotoxicity
ANTIMETABOLITES	<p>Folic acid: (Aminopterin, Methotrexate, Pemetrexed, Raltitrexed)</p> <p>Purine: (Cladribine, Clofarabine, Fludarabine, Mercaptopurine, Pentostatin, Thioguanine)</p> <p>Pyrimidine: (Cytarabine, Decitabine, Fluorouracil/Capecitabine, Floxuridine, Gemcitabine, Enocitabine, Sapacitabine)</p>	<ul style="list-style-type: none"> • Mild toxicity • Block DNA synthesis • Cell cycle specific (S phase, DNA synthesis) • Possibly more toxic on the growing fraction of the follicle pool at preantral stage and onward due to their higher mitotic rates and metabolic demands
ALKYLATING AGENTS	<p>Nitrogen mustards: (Chlorambucil, Chlormethine, Cyclophosphamide, Ifosfamide, Melphalan, Bendamustine, Trofosfamide, Uramustine)</p> <p>Nitrosoureas: (Carmustine, Fotemustine, Lomustine, Nimustine, Prednimustine, Ranimustine, Semustine, Streptozocin)</p> <p>Platinum (alkylating-like): (Carboplatin, Cisplatin, Nedaplatin, Oxaliplatin, Triplatin tetranitrate, Satraplatin)</p> <p>Alkyl sulfonates: (Busulfan, Mannosulfan, Treosulfan)</p> <p>Hydrazines: (Procarbazine)</p> <p>Triazenes: (Dacarbazine, Temozolomide)</p> <p>Aziridines: (Carboquone, ThioTEPA, Triaziquone, Triethylenemelamine)</p>	<ul style="list-style-type: none"> • Alkylating chemotherapeutics are the most gonadotoxic agents. • Targets cells at different stage of cell cycle (not cell cycle specific). They exert selectively more toxicity on resting primordial follicles (cyclophosphamide). • High-dose cyclophosphamide (200 mg/kg) is frequently used as conditioning therapy before bone marrow transplantation (BMT). • Used as the first line therapy for leukemia, lymphoma and other pediatric tumors.
SPINDLE POISONS MITOTIC INHIBITOR	<p>Taxane: (Docetaxel, Larotaxel, Ortataxel, Paclitaxel, Tesetaxel).</p> <p>Vinca: (Vinblastine, Vincristine, Vinflunine, Vindesine, Vinorelbine), Ixabepilone</p>	<ul style="list-style-type: none"> • Less cytotoxic than alkylating agents and platinum group. • Taxanes function as mitotic inhibitor by stabilizing microtubules and as a result, interfering with the normal breakdown of microtubules during cell division. • The vinca alkaloids inhibit assembly of microtubule structures. Disruption of the microtubules arrests mitosis in metaphase. Therefore, the vinca alkaloids affect all rapidly dividing cell types including cancer cells, but also those of intestinal epithelium and bone marrow. • No ovarian toxicity was documented in a small number of women receiving vincristine [39]
CYTOTOXIC ANTITUMOR ANTIBIOTICS	<p>Anthracycline family: (Aclarubicin, Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, Amrubicin, Pirarubicin, Mitoxantrone, Pixantrone, Valrubicin, Zorubicin)</p>	<ul style="list-style-type: none"> • Anthracyclins inhibit DNA and RNA synthesis by intercalating between base pairs of the DNA/RNA strand, thus preventing the replication of rapidly-growing cancer cells. • They also create iron-mediated free oxygen radicals that damage the DNA and cell membranes. • They follow alkylating and platinum compounds in ovarian toxicity.

Table 2—Chemotherapy drugs. (*devami*)

Category	Group	Gonadotoxicity
	Streptomyces (Actinomycin, Bleomycin, Mitomycin, Plicamycin) - Hydroxyurea	<ul style="list-style-type: none"> • They inhibit transcription by binding DNA at the transcription initiation complex and preventing elongation by RNA polymerase. • Their gonadal toxicity profiles are similar to anthracyclins.
TOPOISOMERASE INHIBITORS	Camptotheca: (Camptothecin, Topotecan, Irinotecan, Rubitecan, Belotecan), Podophyllum: (Etoposide, Teniposide)	They form a ternary complex with DNA and the topoisomerase II enzyme, preventing re-ligation of the DNA strands. This causes errors in DNA synthesis and promotes apoptosis of the cancer cell. Limited data suggest moderate ovarian toxicity. [40-42]
MONOCLONAL ANTIBODIES	Receptor tyrosine kinase: (Cetuximab, Panitumumab, Trastuzumab) - CD20 (Ritutumomab) Other (Alemtuzumab, Bevacizumab, Edrecolomab, Gemtuzumab)	No data on ovarian toxicity
TYROSINE KINASE INHIBITORS	Axitinib, Bosutinib, Cediranib, Dasatinib, Erlotinib, Gefitinib, Imatinib, Lapatinib, Lestaurtinib, Nilotinib, Semaxanib, Sorafenib, Sunitinib, Vandetanib	No data on ovarian toxicity
CYCLIN DEPENDENT KINASE INHIBITORS	Alvocidib, Seliciclib	No data on ovarian toxicity
OTHERS	Fusion protein (Aflibercept), Denileukin diftitox	No data on ovarian toxicity
PHOTOSENSITIZERS	Aminolevulinic acid, Etoposide, Methyl aminolevulinate, Porfimer sodium, Talaporfin, Temoporfin, Verteporfin	No data on ovarian toxicity
UNGROUPED	Retinoids (Alitretinoin, Tretinoin), Anagrelide, Arsenic trioxide, Asparaginase (Pegaspargase), Atrasentan, Bortezomib, Carmofur, Celecoxib, Demecolcine, Elesclomol, Elsamitruicin, Etoglucid, Lonidamine, Lucanthone, Masoprocol, Mitobronitol, Mitoguazone, Mitotane, Oblimersen, Omacetaxine, Sitimagene ceradenovec, Tegafur, Testolactone, Tiazofurine, Tipifarnib, Vorinostat	No data on ovarian toxicity

Radiation

Direct action of radiation on DNA is the predominant mechanism of damage for particle radiation such as neutrons and particles. There are also indirect actions that come from the interaction of radiation with other substances in the cell such as water lea-

ding to formation of free radicals and DNA damage. This mechanism is particularly true for sparsely ionizing radiation such as x-rays. Gonadal damage occurs by direct exposure to radiation such as in the case of pelvic or low abdominal or the lumbo-sacral spinal irradiation [2]. Also scatter radiation may cause

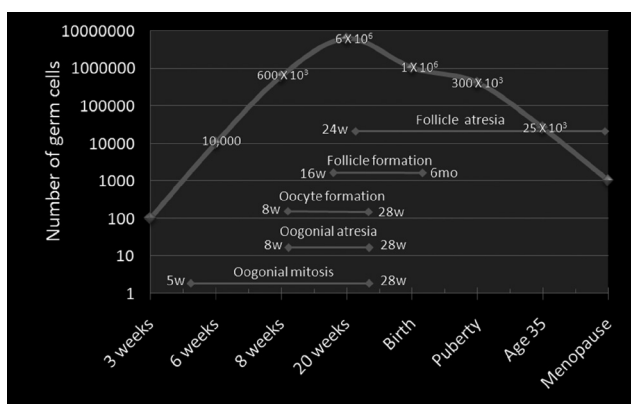
significant damage even if the gonads are outside the radiation field. The risk of premature ovarian failure is higher with increasing radiation doses. Single dose appear to be more toxic effects than fractionated dose [10]. Different from chemotherapy, uterine function is also often compromised by radiation. Radiation-induced damage to uterine vascular and muscular structures result in decreased uterine blood flow, reduced uterine volume, decreased endometrial thickness, and loss of distensibility [2]. These changes can potentially lead to adverse pregnancy outcomes such as miscarriages, still births, preterm deliveries in the survivors of childhood cancers [11]. Craniospinal irradiation is another form of radiotherapy commonly used for the treatment of pediatric brain and spinal cord tumors. Even though there is no direct impact of this form of radiotherapy on the ovaries and the uterus, its use may be associated with abnormalities in the reproductive function by causing abnormal pubertal timing and other endocrinopathies.

Germ Cell Dynamics in the Ovary

Primordial germ cells (PGC) are the embryonic precursors of oocytes. They are differentiated from the proximal epiblast adjacent to the extra-embryonic ectoderm under the influence of the signals from extra embryonic ectoderm-derived bone morphogenetic proteins (BMP) 4 and 8b, and extra embryonic endoderm derived BMP2 [12-13]. They first appear as a cluster of approximately 100 cells in the endoderm of the dorsal wall of the yolk sac near the allantois between the 3rd and 4th weeks of gestation in the human [14]. They then migrate to the hindgut and dorsal mesentery during the 4th and 5th weeks of gestation, respectively [14]. By the 7th week of gestation, colonization of gonadal tissue with germ cells is complete. Germ cells are essential for the formation and maintenance of the ovary. In their absence the gonad degenerates into cord-like structures [15]. Once the PGC have arrived in the gonad in the gonads they exhibit more extensive proliferation such that their number rapidly increases to 600,000 at the 8th week from merely 10,000 at the 6th week of gestation. With rapid mitotic activity their number further rises to 6 million at the 20th week of gestation; thereafter the rate of oogonial mitosis progressively declines and ends at about 28 weeks with almost equally increasing rate of oogonial atresia, which peaks at 20 weeks

of gestation (Figure-2). In a newborn ovary approximately one million germ cells are present of which, only three or four thousands will remain at puberty. The vast majority of these cells will undergo atresia with less than one percent (three hundreds) reaching the ovulatory state until menopause whereafter only 1000 will remain in the ovary [16-17]. This is the physiological form of atresia and occurs gradually in years. However, in the pathological form of follicle atresia as occurs after exposure to cytotoxic chemotherapy regimens and radiotherapy follicle loss occurs in an accelerated, premature and massive manner causing early exhaustion of the follicle pool and ovarian failure.

Primordial follicles represent the earliest stage of follicular development. Their oocytes are surrounded by a single layer of flattened pre-granulosa cells. Ovarian reserve is determined by the number of quiescent primordial follicles in the ovary. Dormant primordial follicles constitute 90% of follicle pool in the human ovary while the remaining 10% belongs to growing follicles at primary stage and beyond [17]. Since activation of primordial follicles for growth is not under endocrine control of gonadotropins they do not express FSH receptor [18], or produce antimüllerian hormone (AMH) [19]. Therefore there is no hormonal or any other marker of them that guide cli-



Figur 2–The saga of female germ cells in human. Once specified as a cluster of 100 cells, germ cells begin to migrate, proliferate and colonize the prospective gonads. With exponential growth their number dramatically increases from 600 000 at 8 weeks, and to 6–7 million at 20 weeks of gestation. Thereafter their number progressively declines after 20th weeks owing to atresia. It remains an unsolved puzzle in reproductive biology why millions of germ cells are wastefully lost in order to select only 300–400 (<1%) for ovulation. bulunmuştur.

nicians to predict their number in the ovary. Any toxic insult that preferentially targets primordial cells such as alkylating cancer drugs (cyclophosphamide, busulfan and melphalan) causes more detrimental effect on ovarian reserve leading to either a decrease in reproductive life span or premature ovarian failure. If the loss of ovarian function develops during or shortly after the completion of cancer therapy, it is termed acute ovarian failure (AOF). For survivors who retain ovarian function after the completion of cancer treatment, a subset will go on to experience menopause before age 40 yr and is classified as having premature menopause [20].

Acute Ovarian Failure (AOF)

We know that AOF develop at least in a subset of survivors of pediatric and adolescent cancers whereas the precise incidence of AOF is not known, and data concerning its risk factors are limited. The first report of the Childhood Cancer Survivor Study was published in 2006 [20]. The study 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. The second report was published in 2009 with longer durations of follow-up and reviewed the frequency of acute ovarian failure, premature menopause, live birth, stillbirth, spontaneous and therapeutic abortion and birth defects in the participants in the Childhood Cancer Survivor Study [21]. The results has shown that acute ovarian failure (AOF) occurred in 6.3% of eligible survivors. Exposure of the ovaries to high-dose radiation (especially over 10 Gy), alkylating agents and procarbazine, at older ages, were significant risk factors for AOF [21]. Young patients with good ovarian reserve are more likely to retain some residual ovarian function after exposure to cytotoxic cancer therapies than older counterparts with diminished ovarian reserve. It has been documented that the lethal dose 50, the ra-

diation dose required to kill 50% of oocytes of the human oocyte is 2 Gray (Gy) [10]. The ovary of younger individuals is more resistant to permanent damage from irradiation than is the ovary of older individuals due to a higher number of primordial follicles in younger ovaries [7, 22-23]. For instance, 6 Gy may be sufficient to produce irreversible ovarian damage in women older than 40 years of age in contrast to 10 to 20 Gy doses needed to induce permanent ovarian failure in the majority of females treated during childhood [24-25]. This is because younger patients harbor more primordial follicles in their ovaries; therefore, they are more likely to retain some residual ovarian function after radiotherapy than do older patients. Sadly, the most devastating effect of radiation on the ovary occurs in patients who receive a stem cell transplant with high-dose total body irradiation (TBI). One study showed that almost all of the patients who had undergone a marrow transplant with TBI after age 10 years developed acute ovarian failure, whereas approximately 50% of girls who had received a transplant before age 10 years suffered acute loss of ovarian function [26]. TBI given as a single dose or fractionated (10–15 Gy), is often used in combination with gonadotoxic cyclophosphamide or melphalan. The use of cyclophosphamide in conjunction with radiation further increases the extent of the damage as exemplified by a study showing that all of 144 patients receiving TBI with cyclophosphamide for bone marrow transplantation developed amenorrhea in the first 3 years. Return of menses occurred 3 to 7 years post-transplant only in 9 patients; all were younger than age 25 [27]. Similarly 5 of ten Fanconi anemia patients (median age at transplantation was 12 years (range 5-17 years) undergoing hematopoietic stem cell transplantation developed signs of ovarian failure during follow-up [28]. Based on the available data it is safe to conclude that most recipients of allogeneic hematopoietic stem cell transplantation suffer from infertility owing to gonadal damage from myeloablative conditioning with chemotherapy with and without irradiation.

Premature Menopause

Premature menopause is another form of gonadal failure characterized by 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. Using data from the Childhood Cancer Survivor Study, ovarian function was analyzed 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 [29]. 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% [29]. Later 2009 report of the study confirmed the findings showing the same incidence of premature nonsurgical menopause (PM) (8% of participants versus 0.8% of siblings (rate ratio = 13.21; 95% CI, 3.26 to 53.51; $P < .001$) [21]. A cohort-study conducted among 518 female 5-year Hodgkin lymphoma (HL) survivors, aged 14 to 40 years (median: 25 years) showed that after a median follow up of 9.4 years, 97 women had reached menopause before age 40 years. Chemotherapy was associated with a 12.3-fold increased risk of premature menopause compared with radiotherapy alone. Treatment with MOPP (mechlorethamine, vincristine, procarbazine, prednisone)/ABV (doxorubicine, bleomycine, vinblastine) significantly increased the risk of premature menopause (hazard ratio [HR]: 2.9), although to a lesser extent than MOPP treatment (HR: 5.7). Alkylating agents, especially procarbazine (HR: 8.1) and cyclophosphamide (HR: 3.5), showed the strongest associations. Ten years after treatment, the actuarial risk of premature menopause was 64% after high cumulative doses ($> 8.4 \text{ g/m}^2$) and 15% after low doses ($\leq 4.2 \text{ g/m}^2$) of procarbazine [30]. Notably, the cumulative risk of menopause at age 40 years did not differ much according to age, but time to premature menopause was much longer in women treated at early ages confirming that women who were older at the time of first treatment experienced premature menopause sooner after treatment than younger women since younger patients have higher ovarian reserve and are therefore more likely to retain some residual

ovarian function after chemotherapy. In contrast to alkylating regimens, chemotherapy with doxorubicin (Adriamycin), bleomycin, vinblastine, and dacarbazine (ABVD) appear to be less toxic. ABVD regimen remains a common and successful first-line treatment protocol for young people with Hodgkin lymphoma. Studies show comparable follicle densities in the ovaries in patients undergoing ovarian tissue freezing before and after receiving ABVD regimen [9, 31]

Markers of Ovarian Reserve and Function

It is of crucial importance to obtain accurate information on the gonadotoxicity of different cancer treatment regimens to provide the best approaches for fertility preservation. Data on the gonadotoxic potential of cancer drugs are largely collected from 2 important sources; animal studies and clinical trials. Several animal studies, mainly in rodents, showed individual gonadotoxicity of certain cancer drugs such as cyclophosphamide [8]. However, the major drawback of utilizing animals is the differences in ovarian physiology, the mechanisms of action of cancer drugs, or different thresholds of gonadal toxicity between human and the animal tested. The most accurate information of gonadotoxicity on the human ovary can be obtained by real-time quantitative analysis of primordial follicle counts using histomorphological methods in ovarian samples, which necessitates an operation. It cannot be done in clinical settings for ethical and practical reasons. Moreover, as new agents are introduced to adjuvant setting, their long-term impact on the human ovary is extremely difficult to determine from short-term studies.

In clinical studies, the magnitude of the impact of chemotherapy on the human ovary is determined by assessing either menstrual function or the markers of ovarian reserve or both in patients receiving that chemotherapy regimen. It should be emphasized that menstrual status is a crude marker of fertility, as shown previously in patients who were still menstruating despite their critically elevated follicle stimulating hormone (FSH) levels and diminished ovarian reserve [32]. Therefore the presence or return of menstruation at the end of treatment does not necessarily connote normal fertility or reproductive life span. Furthermore, current modern cancer treatments commonly employ multiagent chemotherapy drugs precluding assessment of individual gonadoto-

xicity of each drug in a combination regimen. Ovarian reserve markers such as FSH, estradiol, and anti-Mullerian hormone measurements (8,9), as well as antral follicle counts (10), can give a better estimate of ovarian reserve before and after chemotherapy. Indeed, most data on markers of ovarian reserve and response arise from the clinical context of improving prediction of outcome during assisted reproduction, but has also been translated to the effects of chemotherapy on gonadal function. Unfortunately, in contrast to a preponderance of such studies in adult cancer patients evaluating the impact of chemotherapy regimens with these markers, there are only a few conducted in pediatric cancer patients. Among those antimullerian hormone (AMH) appears to provide a more accurate estimation of ovarian reserve. AMH is produced by the granulosa cells of growing preantral and small antral follicles as a dimeric glycoprotein [19]. In fetal ovaries, AMH is first detected at 36 weeks of gestation in granulosa cells of developing preantral follicles [33] and reaches its highest levels in puberty and becomes undetectable after menopause [34-35]. AMH has recently emerged as an important marker of ovarian reserve. A growing body of evidence now suggests that AMH can be used both for assessment of ovarian reserve and prediction of IVF outcome [17]. A recent study conducted in 185 survivors of childhood cancers (median age at the time of treatment was 5.8 years (range 0.1–16.8 year) and median age at the time of AMH assessment 25.5 year (range 17.0–47.4 year) has shown that AMH levels were lower than the 10th percentile of normal values in 27%(49/182) of the survivors. In addition, 43%(79/182) had AMH levels lower than 1.4 mg/l, a previously established cut-off value which predicts ongoing pregnancy after assisted reproduction. Subgroup analysis revealed that survivors treated with three or more procarbazine (alkylating agent) containing chemotherapy cycles or with abdominal or total body irradiation had significantly lower AMH levels than controls (median 0.5 mg/l; $P = 0.004$ and median, 0.1 mg/l; $P < 0.001$, respectively) [36]. AMH can be used to identify subgroups of CCS at risk for decreased fertility or premature ovarian failure

Another study analyzed FSH, LH, estradiol and anti-mullerian hormone (AMH) levels on days 3-5 of a menstrual cycle in thirty three cancer survivors in mean age 19.1+/-4.7 years treated in age 12.0+/-5.6

years for Hodgkin Lymphoma (HL) (n=16), nephroblastoma (n=7), soft tissue sarcoma (n=4), germinal tumor (n=3), neuroblastoma (n=2), histiocytosis (n=1). Infradiaphragmatic radiotherapy was needed in 16 patients (10 treated for HL) in comparison to healthy controls.

Patients treated for HL with chemo- and radiotherapy presented higher FSH levels than controls (8.53+/-3.25 vs. 5.8+/-2.03 mIU/ml; $p=0.045$). Mean AMH levels were lower in all patients that received radiotherapy for the infradiaphragmatic region (17.19+/-14.84 pmol/l) than in controls (29.40+/-13.2 pmol/l; $p=0.037$). Particular analysis of all cases showed higher (>2 SD) FSH levels in 8 patients: 5 patients treated for HL with radiotherapy and higher total doses of procarbazine, nitrogen mustard and vinblastine; 2 patients treated for soft tissue sarcoma and one patient for Wilms tumor (all received radiotherapy). Lowered AMH levels were found in 8 patients treated with chemo- and radiotherapy (4 - for HL, 2 - for Wilms tumor and 2 - for soft tissue sarcoma) [37]. Similar results were also reported by others which showed lower inhibin B levels than controls (median, 94 vs. 111 pg/ml; $P = 0.03$) and higher estradiol levels (median, 0.12 vs. 0.08 pM; $P = 0.04$) in 100 survivors of childhood cancers. When multiple linear regression analysis was performed to predict the total antral follicle number per ovary, and it showed a reduced number with ovarian irradiation ($\beta = -0.40$, $P < 0.001$), alkylating chemotherapy ($\beta = -0.25$, $P = 0.01$), older age at diagnosis ($\beta = -0.25$, $P = 0.01$), and longer time period off treatment ($\beta = -0.19$, $P = 0.044$). One in every six female survivors may develop premature ovarian failure[38]. All these studies underscore the impact of chemotherapy (especially alkylating category) and radiation exposed during childhood on ovarian reserve. Therefore every adult survivor of pediatric cancers and the parents of the children with cancer should be informed about the risks of premature ovarian failure and other reproductive harms caused by chemotherapy and radiation.

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